(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 May 2002 (02.05.2002)

PCT

(10) International Publication Number
WO 02/34110 A2

(51) International Patent Classification[7]: A61B

(21) International Application Number: PCT/US01/32564

(22) International Filing Date: 18 October 2001 (18.10.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/697,934 27 October 2000 (27.10.2000) US

(71) Applicant *(for all designated States except US)*: **AKZO NOBEL N.V.** [NL/NL]; N.V. Organon, Weth. V. Eschstraat 1, P.O. Box 20, NL-5340 BH Oss (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **TEJIDOR, Liliana** [US/US]; 10440 Leslie Drive, Raleigh, NC 27615 (US). **FISCHER, Timothy, J.** [US/US]; 11530 N. Charoleau Drive, Tucson, AZ 85737 (US). **BAGLIN, Trevor** [GB/GB]; 2 Royston Road, Whittlesford, Cambridgeshire CB2 4NW (GB).

(74) Agent: **MYERS BIGEL SIBLEY & SAJOVEC**; P.O. Box 37428, Raleigh, NC 27627 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/34110 A2

(54) Title: METHOD OF DETERMINING GLOBAL COAGULABILITY AND HEMOSTATIC POTENTIAL

(57) Abstract: A method is disclosed for determining if a patient is hypercoagulable, hypocoagulable or normal. The test involves providing a test sample from the patient and initiating coagulation in the sample in the presence of an activator, which is added to the sample in an amount which will result in intrinsic tenase-dependent fibrin. Then the formation of the intrinsic tenase-dependent fibrin polymerization is monitored over time so as to derive a time-dependent profile, with the results of the fibrin polymerization monitoring determining whether the patient is hypercoagulable, normal or hypocoagulable. The coagulation activator is added in an amount that triggers a thrombin explosion that is dependent on the propagation phase and amplification pathways. In this way, a single assay can assess the hemostatic potential of a sample.

Method of Determining Global Coagulability and Hemostatic Potential

FIELD OF THE INVENTION

The present invention is related to US patent 5,646,046 to Fischer et al and US patent 6,101,449 to Givens et al, the subject matter of each being incorporated herein by reference. The invention is directed to a method for determining whether a patient is hypercoagulable, hypocoagulable or normal in a single test on a sample from the patient. The invention allows for globally assessing both the hypercoagulable potential and hypocoagulable potential of a patient in a single assay.

BACKGROUND OF THE INVENTION

Hemostasis is the entire physiological process of maintaining blood in a fluid state within intact blood vessels and preventing excess blood loss by arresting flow via the formation of a hemostatic plug. Normal hemostasis is maintained by tightly regulated interactions of the blood vessel wall, blood platelets and blood plasma proteins. Under normal conditions there is a delicate balance between the individual components of the hemostatic system. Any disturbances in this hemostatic balance, the hemostatic potential, could result in bleeding or thrombosis, Figure 1. By "hemostatic potential" we mean the ability to maintain a balance between procoagulant and anticoagulant states, as measured by fibrin polymerization, when coagulation is initiated by a trigger or activator.

A thrombotic tendency (thrombophilia) results from the generation of excess thrombin activity and increased fibrin polymerization and clot formation (hypercoagulability) while a bleeding tendency (hemophilia) results from insufficient thrombin generation and reduced fibrin polymerization and clot formation (hypocoagulability). There is as yet no

single laboratory parameter that is increased in all forms of hypercoagulability and decreased in all forms of hypocoagulability. This is in part due to factors other than plasma that play a part in hemostasis. As described above, these other factors include the blood vessel wall and platelets. However, large proportions of the hemostatic disorders are related to defects or deficiencies in the blood proteins that constitute the coagulation system. These proteins are responsible for the stabilization of the platelet plug by the formation of fibrin. Therefore, a global measure of the plasma contribution to coagulation would facilitate the investigation and management of patients with altered hemostasis.

Thrombophilia and haemophilia can be either congenital or acquired. The congenital forms have a genetic basis and are therefore not readily corrected. The acquired forms generally result from environmental changes, often the effect of drugs, and are therefore susceptible to manipulation. For example a normal individual given warfarin develops acquired haemophilia, stopping the warfarin abolishes the condition. A normal individual given high dose estrogen develops acquired thrombophilia, stopping the estrogen abolishes the condition. The fundamental basis of both the congenital (genetic) and acquired (environmental) thrombophilias and haemophilias is a change in either the amount or activity of one or more key components of the coagulation pathway. For example the most commonly recognized hereditary form of thrombophilia is a mutation in the factor V gene which results in the production of a structurally altered factor V protein (Factor V Leiden) that is resistant to enzymatic cleavage by protein C, a critical regulatory component. Classical Haemophilia A is due to a mutation in the factor VIII gene which results in either reduced production of factor VIII, or production of a structurally altered factor VIII protein that does not function correctly. In contrast to the congenital thrombophilias and haemophilias the acquired forms do not result from altered structure but rather alteration of the amount of a key component, typically more than one at a time. For example the thrombophilic effect of oestrogen is due to the composite effects of a rise in factors XI, IX, VIII, II and fibrinogen and a reduction in

the anticoagulant protein S. The haemophilic effect of warfarin is due to a reduction in factors II, VII, IX and X. Figure 2 illustrates the various states of coagulability and lists examples of assays used to assess the degree or presence of an imbalance. There is currently not an assay that can be used to assess both hyper and hypocoagulability simultaneously. This is due in part to the complexity of the coagulation process, the interdependence of the various components and the identification of a means to monitor the hemostatic potential of the entire coagulation system. Figure 3 presents an overview of the coagulation process. The process can be divided into four dependent phases, (1) the initiation phase, (2) the propagation phase, (3) the amplification phase and (4) the polymerization phase. All of the phases are affected by regulatory and feedback processes referred to as anticoagulant pathways.

Initiation or triggering of coagulation occurs by exposure of tissue factor due to vascular damage, plaque rupture or monocyte expression as a result of inflammation. Trace amounts of FVIIa and tissue factor form the extrinsic Xase complex. This complex enhances the catalytic activity of VIIa towards factors X and IX resulting in the formation of the active enzymes Xa and IXa. Factor Xa generated by the extrinsic Xase complex forms a small amount of thrombin (IIa). The thrombin generated is capable of activating small amounts of the cofactors VIII and V. In vivo, the extrinsic Xase complex is quickly inactivated by Tissue Pathway Factor Inhibitor, TFPI, via the formation of a quaternary complex consisting of TF, VIIa and Xa. Under physiological conditions the extrinsic Xase generates only picomolar amounts of thrombin.

During the propagation phase of coagulation the role of the extrinsic Xase is minimized and Factor Xa is alternatively generated by the complex of the enzymes IXa and its cofactor VIIIa. This enzyme complex is referred to as intrinsic Xase. Formation of the Xa by the intrinsic Xase complex is approximately 50 fold more efficient than the extrinsic Xase. Factor Xa and its activated cofactor, FVa, form a complex on the surface of activated platelets. This is an efficient catalyst for the conversion of

prothrombin to thrombin, referred to as the prothrombinase complex. Thrombin formed via the intrinsic Xase complex is capable of amplifying its own production by positive feedback (activation). Thrombin activates Factors VIII and V and Factor XI activation leads to further production of the enzymatic component of intrinsic Xase (Factor IXa). Normal thrombin production is highly regulated and localized. TFPI neutralizes the trigger for thrombin generation. Active proteases (IIa, Xa, IXa) must be inactivated by protease inhibitors to avoid disseminated thrombosis. One of the most significant of these inhibitors is antithrombin III (ATIII). Both thrombin and Xa, and to a lesser extent IXa released from membrane surfaces, are rapidly inhibited by ATIII. Thrombin can also bind non-damaged sub-endothelium via a receptor molecule, Thrombomodulin (TM). The formation of the IIa/TM complex changes the substrate specificity of thrombin from a procoagulant to an anticoagulant. Thrombin bound to TM is a potent activator of Protein C, converting it to the active enzyme Activated Protein C (APC). APC together with its cofactor protein S cleaves activated cofactors FVIIIa and FVa yielding their inactive forms, FVIIIi and FVi. Thrombomodulin also accelerates the inactivation of thrombin by ATIII.

The formation of thrombin leads ultimately to cleavage of fibrinogen to form fibrin. During the polymerization phase cross-linking of soluble fibrin strands is mediated by Factor XIIIa, an enzyme generated by thrombin activation. The thrombin-TM complex activates the procarboxypeptidase thrombin activated fibrinolysis inhibitor (TAFI). Thus thrombin plays a role during this phase by both influencing the architecture and stabilization of the fibrin clot. Thrombin is a key enzyme and effector of the coagulation process. Thrombin is both a potent procoagulant and anticoagulant. However, it is thrombin's ability to cleave fibrinogen and its contribution to fibrin polymerization events that are critical to maintaining stasis.

Clot initiation, often referred to as clotting time, occurs at the intersection between the initiation and propagation phases when only

approximately 5% of thrombin has been formed. The majority of the thrombin formed is generated after the initiation of fibrin polymerization, thus the rate of fibrin polymerization is a more sensitive indicator of the dynamics of coagulation. Changes in the propagation phase, amplification phase and anticoagulant pathways alter the rate of thrombin generation and the impact of thrombin availability on rate of fibrin polymerization. Recent studies by Cawthern et al. (1998) suggested that measurement of this thrombin is more informative than clotting time in assessing the pathophysiology of hemophilias. However these investigators measured thrombin by looking at the kinetics of formation of the thrombin-antithrombin complex (indictor of thrombin generation) and formation of fibrinopeptide A (indicator of fibrinogen cleavage) and not by measuring the kinetics of fibrin polymerization. Variations in concentration or quality of the fibrinogen or fibrin strands can only be measured as a function of the actual polymerization process. Assays currently used to assess variations in the coagulation process typically can only assess variations in one or two phases. These assays measure events independently and therefore negate or eliminate the ability to detect variations in the other phases or interactions between the various phases.

Assays associated with the assessment of bleeding risk include the Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), Thrombin Time (TT) and Fibrinogen (Fib) assays (figure 2). These assays are based on the addition of potent activators of the coagulation process and thus are only abnormal when major defects are present. These assays are not designed to detect the composite effect of multiple minor alterations. For example in the PT test, which utilizes a very high concentration of a tissue extract, called thromboplastin, and calcium are added to citrated plasma. Whole blood is mixed with citrate when the blood sample is taken. The citrate binds the calcium and "anticoagulates" the blood as calcium ions are required for assembly of the tenase and prothrombinase complexes. The blood sample is then centrifuged and the plasma is separated. When calcium is added back, the tenase (or Xase) and prothrombinase complexes can form and thrombin can be generated.

The source of tissue factor is the thromboplastin. However, the concentration of tissue factor is extremely high (supraphysiological) and so only the initiation phase of thrombin generation is required. The propagation and amplification phases are bypassed. The prothrombin time is therefore insensitive to many changes in the coagulation pathway and is incapable of detecting hypercoagulability. Assays based on diluted thromboplastin have been formulated to aid in the diagnosis of patients with antiphospholipid syndrome (APS). In these methods the thromboplastin together with the phospholipids are diluted to enhance the sensitivity of the PT to the presence of antiphospholipid antibodies. The dilute PT clotting time is prolonged in APS due the unavailability of phospholipid surfaces and therefore the assay is phospholipid dependent instead of TF dependent.

Assays associated with the assessment of a hypercoagulable state (figure 2) include the Thrombin Anti-Thrombin Complex (TAT), Prothrombin fragment F1.2, PAI 1, APCr and D-dimer. These assays are designed to measure a specific marker or product of the coagulation process. For example, the measurement of elevated levels of D-dimer indicates that the clotting process has been activated. However, there is no way of determining whether the D-dimer was being produced as a product of the normal healing process or if there is an underlying hypercoagulable risk. The hypercoagulable state cannot be globally assessed by a single assay but currently requires a battery of tests. A global assay for the assessment of hemostatic potential would be able to identify an imbalance utilizing a single assay principle that is sensitive to defects, singular or in combination. The assay would also be sensitive to effects of intervention to restore the hemostatic balance.

Recognising the limitations of the screening assays available for hypcoagulable assessment and the battery of assays required for hypercoagulable assessment, others have tried to develop global tests. These tests were designed to be sensitive to the amount of the biological components and their interactions, as well as measure the dynamics of thrombin generation including regulation. The thrombin generation curve

was described more than 30 years ago as a measure of the thrombin generating potential of plasma. A modification of the thrombin generation curve has been described with quantification of thrombin with a exogenously added chromogenic substrate. This has been called the endogenous thrombin potential (ETP). The assay assumes that there is a direct correlation between endogenous thrombin potential measured via an exogenously added artificial substrate and the assessment of a hemostatic imbalance. The use of an artificial substrate instead of thrombin's natural substrate, fibrinogen, ignores the effects of variations in fibrinongen concentration and fibrinogen configuration. Thrombin is a cleavage product from the proteolysis of Prothrombin, a serine protease. Thrombin then cleaves fibrinogen, its natural substrate, resulting in soluble fibrin monomers that are crossed linked via FXIIIa to formed crossed linked polymerized clots. Thrombin is a highly regulated molecule that possesses both procoagulant and antithrombotic behavior. Additionally, there are numerous substrates that inactivate thrombin before it can cleave fibrinogen. In addition to not directly measuring the ability to form a clot the ETP assay has several other major limitations. Limitations of the test include:

1. The plasma sample must be defibrinated, typically with a snake venom. Defibrinating snake venoms activate FX and they also cleave the chromogenic substrate used to quantitate thrombin. This can cause a variable over-estimate of the thrombin potential.
2. The plasma sample is considerably diluted in order to prolong the dynamics of thrombin generation. This results in a non-physiological regulation of the thrombin explosion.
3. The technique involves multiple subsampling at specified timepoints. For example, a computer linked pipeting device designed in order to terminate thrombin activity in the subsamples exactly at a specified time. It is possible to perform the assay manually but it is beyond the ability of many technologists and requires considerable skill. The test cannot be automated on standard clinical laboratory coagulometers.

4 The formation of thrombin-α2 macroglobulin complex leads to over-estimation of the thrombin potential. A complex mathematical manipulation of the results to approximate it to the true thrombin potential is therefore required.

5 Does not take into account the rate or ability of thrombin to cleave fibrinogen.

Duchemin et al. described a further modification of the ETP where the protein C pathway is assessed by adding exogenous thrombomodulin. This method was also modified to take into account proteins that modulate anticoagulant activity, including antithrombin III. Like ETP, this modified assay is designed to only measure thrombin generation and not the effects of thrombin, i.e. dynamic clot formation.

Other investigators have attempted to design assays sensitive to the composite of biological components of the coagulation process and their interactions. One such example is described by Kraus (Canadian application 2,252,983). The method is however limited to determining the anticoagulant potential of a sample by adding thrombomodulin and thromboplastin in a coagulation test. In the described method the emphasis is on dilutions of thromboplastin such that thrombin is produced at a rate slow enough to enable sufficient activation of protein C during the measuring time of the coagulation apparatus. A disadvantage of this method is that because it depends on clot time, the amount of thromboplastin is more restrictive and higher concentrations are required to compensate for increases in clotting time when thrombomodulin is added. Because the method described is aimed at assessing anticoagulant potential and not global hemostatic potential the assay is not sensitive to defects in the propagation and amplification phases, the kinetics of clot polymerization or to the interrelationships between the factors responsible for thrombin generation.

The present invention however assesses both the anticoagulant and procoagulant potential of a blood sample. Furthermore, the present invention's sensitivity can be enhanced by using more dilute coagulation activator, more dilute than has previously been used, since the endpoint

method is not restricted to clot time but analysis can be conducted for the entire dynamic coagulation process as measured by evaluating kinetic parameters of the optical data profile. Analysis of more than simply clot time can be accomplished even when very weak and unstable clots are formed.

Variations in the amplification and/or propagation phases will reduce or alter the rate of generation of thrombin and thus impact the rate of fibrinogen cleavage and ultimately the rate of fibrin polymerization. Because the present invention can measure the rate of fibrin polymerization throughout the dynamic coagulation process, it measures the clinically important thrombin that is generated after clotting time.

Other prior art (Mann et. al.) assesses coagulation problems by taking a series of independent and indirect measurements. Thrombin generation is measured as a function of TAT complex formation or the use of a chromogenic substrate and the formation of fibrin as measured by the release of FPA. All of the systems and models to date have been designed to understand a discrete process or interaction of the coagulation process and cannot provide an assessment of the overall hemostatic potential. In contrast, the method of the present invention is designed to not only assess the interplay of the coagulation proteins together with synthetic cell surfaces, it is aimed at capturing this in a dynamic measurement that correlates to clinical outcome. The technology and methods described in the present invention can also be modified to introduce components of the fibrinolytic system as well as cells and flow conditions.

Givens et. al. demonstrated that a model which characterizes the process of clot formation and utilizes parameters in addition to clotting time is sensitive to defects in the clotting proteins. Table 1 describes the parameters defined by Givens et al. and Figures 4 and 5 illustrate how those parameters are determined and how they relate to fibrin polymerization for the PT and aPTT assays. However, this work was conducted utilizing data from the PT and APTT assays, which as discussed earlier, are only sensitive to events associated with the

hypocoagulable state. Additionally, the work described was conducted in the presence of strong clot formation because of the addition of supraphysiological concentrations of tissue factor. Fibrin polymerization is significantly altered in a dilute systems designed for global hemostatic assessment resulting in weak and unstable clot formation. Global hemostatic assessment and new methods for monitoring and quantifying fibrin polymerization are required.

SUMMARY OF THE INVENTION

In order to overcome the deficiencies in the prior art as noted above, a global test of coagulation has now been developed, which is accurate and easy to use. With the present invention, a single test can be used to quantify both hyper- and hypocoagulability. The concept is based on the addition of a minimal concentration of coagulation activator sufficient to trigger but insufficient to result in complete fibrin polymerization so as to allow detection of perturbances in the propagation, amplification and polymerization pathways. In a dilute system, the coagulability (hyper/hypo) of a sample determines the magnitude of the thrombin explosion and the direct and indirect influence that has on the rate of fibrin polymerization. This concept is contrary to an assay system such as the PT, which uses excess amounts of TF (or thromboplastin). In the method of the present invention, therefore, disturbances in the propagation and amplification loops are accessible, whereas in the traditional PT test, these parts of the coagulation pathway are overshadowed by the excessive amounts of Factor IIa produced by the initiation phase.

In one embodiment of the invention, the rate of fibrin polymerization produced by a standardized coagulation activator dilution is then used to indicate if a plasma sample is normal, hyper- or hypocoagulable. In addition, the technique can be used to determine how much the plasma needs to be modified in order to restore coagulability to normal. For example, in the case of hypocoagulability, this might be achieved by

clotting factor replacement or in the case of hypercoagulability, by the addition of a natural anticoagulant or the use of an anticoagulant drug.

In the present invention, at a given coagulation activator dilution, the rate of fibrin polymerization of haemophilia plasmas are less than the rate of polymerization for a normal plasma and the rate of fibrin polymerization of thrombophilia plasmas are greater than that of a normal sample. The rate of fibrin polymerization is sensitive to minor changes in the components of hemostasis even when differences in clotting time cannot be detected. Figure 6 illustrates waveforms from the normal, hypercoagulable and hypocoagulable specimens. The rate of polymerization is affected even though the time of clot initiation is essentially unchanged.

In another embodiment of the present invention, a test is provided that can be used to determine the degree of hyper- or hypocoagulability of a plasma sample. Furthermore, it can be used on samples containing platelets or other cells as a measure of the contribution of cellular components to coagulability. The test, in some embodiments, relies on the use of a standardized dilution of thromboplastin in the presence of an excess of phospholipids with the rate of fibrin formation as the detection endpoint. The test is simple and can be automated on standard laboratory coagulometers. The test in the present invention can be run on a test sample in the absence of the addition of an exogenous substrate, e.g. a chromogenic substrate. The test is sensitive to fibrin concentration and/or configuration.

In a further embodiment of the invention modifications to the components or concentrations of the reagent or endpoint selection are tailored to facilitate the development and/or monitoring of novel pharmaceutical agents. Examples of such applications are inhibitors of initiation of the TF pathway (TFPI, FVIIa inhibitors), inhibitors of thrombin generation such as inhibitors of FXa, (synthetic pentasaccharides) and inhibitors of thrombin activity (direct thrombin inhibitors). Lipid composition, size or concentration can also be modified to tailor the assay towards the development of drugs targeted to the propagation and

amplification pathways. For example, lipid composition can be altered to produce vesicles that maximize Xa generation or alternatively, designed to maximize prothrombinase activity. Thus the efficacy of inhibitors of Xa and those directed at the prothrombinase complex may be assessed. The invention can also be modified to focus on the anticoagulant potential of the plasma by including thrombomodulin, an activator of protein C. Lipid vesicles maximizing the activity of APC could also be added to the reagent. The assay can also be modified to exaggerate a mildly abnormal subpopulation. The consequences of this approach are that severely thrombotic or hemorrhagic samples will exceed the signal to noise ratio and not be measured but subtle differences at the onset of a disease or an earlier indication of effective intervention would be gained. Endpoint selection and ratios derived from comparison to known samples would be exploited to further improve sensitivity and specificity of the reagent modifications. These approaches would therefore be utilized in the drug discovery and drug development processes where assay designed for a global assessment of the hemostatic potential are required.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the consequences of any disturbance in this so-called hemostatic balance or potential.

Figure 2 illustrates the conditions associated with being out of hemostasis and lists examples of assays used to assess the degree or presence of an imbalance.

Figure 3 illustrates the four dependent phases of the coagulation process.

Figure 4 illustrates the optical data from a clotting assay and the first and second derivative calculated from that data.

Figure 5 illustrates where min_2, the time index of min2 (clotting time), min_1, max_2 and delta (proportional to fibrinogen concentration) are located in the optical data profile.

Figure 6 illustrates examples of waveforms for the global screening assay at dilute tissue factor.

Figure 7 illustrates the change in ratio as a function of dilution for a FVIII deficient specimen and a Protein S deficient Specimen.

Figure 8 illustrates ratios of the min-1 values (the maximum rate of fibrin polymerization) for hypocoagulable specimens at three dilutions of rTF compared to the min_1 values of the ratio of the same dilution of a normal plasma.

Figure 9 illustrates ratios of the min_1 values for hypercoagulable specimens at three dilutions of rTF and 10nM thrombomodulin compared to min_1 values of the ratio for the same conditions of a normal plasma.

Figure 10 illustrates the effects on min_1 values of varying tissue factor and thrombomodulin concentrations on results for hypercoagulable, hypocoagulable and normal plasmas.

Abbreviations in the figures are as follows:

Activated Factor IX (FIXa)
Activated Factor V (FVva)
Activated Factor VII (FVIIa)
Activated Factor VIII (FVIIIa)
Activated Factor X (FXa)
Activated Factor XI (FXIa)
Activated Factor XIII (FXIIIa)
Activated Protein C (APC)
Factor II (FII)
Factor IX (FIX or F9)
Factor V (FV)
Factor V Leiden (FVL)
Factor VII (FVII)
Factor VIII (FVIII or F8)
Factor VIII Deficient (FVIII-def)
Factor X (FX)

Factor XI (FXI)
Factor XIII (FXIII)
George King (GK)
HRF (Hemophilia Research Foundation)
Organon Teknika Normal Pool Plasma (OT NPP)
Protein C (PC)
Protein C Deficient (PC Def.)
Protein S (PS)
Protein S Deficient (PS-Def)
Prothrombin Mutation 20210 (PT 20210)
Recombinant Tissue Factor (rTF)
Thrombin or aActivated Factor II (FIIa)
Thrombomodulin (TM)
Tissue Factor (TF)
Von Willebrand Factor (vWF)

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention is directed to a method for determining if a patient or specimen from said patent is hypercoagulable, hypocoagulable or normal in a single test, and comprises the steps of initiating coagulation in a patient's sample *in vitro* in the presence of an activator. Said activator is added to the sample in an amount which will result in intrinsic tenase-dependent fibrin polymerization (involves propagation and amplification loops). Preferably the plasma sample is undiluted thus allowing for sufficient concentrations of all of the endogenous proteases and inhibitors. Formation of the fibrin polymerization is recorded over time so as to derive a graphic time-dependent polymerization profile. This profile will show whether the patient is hypercoagulable, normal, or hypocoagulable by comparing the sample profile with a profile from a known sample.

Preferably, the activator is a thromboplastin, more preferably Tissue Factor (TF). In its most preferred form, the TF is recombinant TF (rTF) that is relipidated with phospholipids, which form liposome vesicles. Preferably phospholipids provide the surfaces to assemble intrinsic Xase and prothrombinase complexes. The phospholipids are present at a concentration, which is not rate limiting to the coagulation process and

remains constant and independent of dilution. These phospholipid vesicles mimic platelet and monocyte surfaces.

Optical data profiles are generated on an automated coagulation analyzer such as the MDA™180 offered by Organon Teknika Corporation. Preferably endpoints such as the time of clot initiation and the rate of polymerization are calculated from the data profiles. More preferably the 1st and 2nd derivatives from the data profile are calculated and the min and max of the derivatives are calculated with respect to value and the associated time index. Most preferably the endpoints are calculated and one or more of the following ratios are calculated using the mentioned endpoints:

Option 1 - Endpoint(s)

Option 2 - Ratio at different dilutions (ratio 1)

$$\frac{\text{Endpoint (z) for dilution (x)}}{\text{Endpoint (z) for dilution (y)}}$$

Option 3 - Ratio of dilutions compared to normal (ratio 2)

$$\frac{\text{Ratio 1 for patient sample}}{\text{Ratio 1 for normal plasma}}$$

Option 4 - Ratio for different reagent formulations

$$\frac{\text{Ratio 2 with formulation (a)}}{\text{Ratio 2 with formulation (b)}}$$

Option 5 - Ratio of different endpoints

$$\frac{\text{Ratio 2 with endpoint (z)}}{\text{Ratio 2 with endpoint (z')}}$$

Option 6 - Ratio of specimen to normal at a given dilution

$$\frac{\text{Endpoint (z) at dilution x for a specimen}}{\text{Endpoint (z) at dilution x for a normal plasma}}$$

Additionally, other ratios, differences or models to normalize the assay can be calculated. The normal plasma can be substituted with any known plasma. Known plasma is defined as a plasma that has been characterized with respect to a condition of the specimen.

Figure 1 illustrates the consequences of any disturbance in this so-called hemostatic balance or potential. Too little hemostasis (decreased platelet function, hypo-coagulation, hyper-fibrinolysis) at the site of injury leads to persistent bleeding, while too much hemostasis (increased platelet function, hyper-coagulation, hypo-fibrinolysis) leads to the formation of an excessive thrombus with vascular obstruction and ischemia.

Figure 2 illustrates the conditions associated with being out of hemostasis and lists examples of assays used to assess the degree or presence of an imbalance.

Figures 3 illustrates the four dependent phases of the coagulation process, (1) the initiation phase, (2) the amplification phase, (3) the propagation phase and (4) the polymerization phase of hemostasis. All of the phases are affected by regulation and feedback processes referred to as anticoagulant pathways.

Figure 4 illustrates the optical data from a clotting assay and the first and second derivative calculated from that data. Table 1 describes a set of parameters calculated from the data and derivatives illustrated in figure 4.

Table 1

Parameter	Description
Slope 1	Initial slope from point A to point B
Delta 1	Amplitude of signal change from point A to point B
Slope 3	Final slope from point D to point E
Delta	Amplitude of signal change
Index Min 1	Time at point C
Min 1	Minimum value of 1st derivative (Rate of change at point C)
Index Max 2	Time at point D
Max 2	Max. value of 2nd derivative (Acceleration at point D)
Index Min 2	Time at point B
Min 2	Minimum value of 2nd derivative

Figure 5 illustrates where min_2, the time index of min2 (clotting time), min_1, max_2 and delta (proportional to fibrinogen concentration) are located in the optical data profile.

Figure 6 contains examples of waveforms for the global screening assay at dilute tissue factor. The APC resistant, , hypercoagulable specimen, generates a waveform that has essentially the same time of clot initiation compared to the normal. However, the rate of fibrin polymerization for the hypercoagulable specimen is significantly greater than that of the normal. The FVIII and FIX deficient hypocoagulable specimens, have only a slightly prolonged time of clot initiation whereas the rates of polymerization are significantly reduced when compared to normal or hypercoagulable specimens.

Figure 7 illustrates the change in ratio as a function of dilution for a FVIII deficient specimen and a Protein S deficient Specimen. The ratio values at 1:50,000 dilution of thromboplastin deviate from the response of the normal plasma. The hypocoagulabe specimen produces ratios that are greater than 1 and the hypercoagulable specimen has ratios that are less than 1 for this endpoint (clot time) / ratio combination. Additionally, the abnormal specimen deviates from normal at different dilutions and in opposite directions.

Figure 8 contains ratios of the min-1 values (the maximum rate of fibrin polymerization) for hypocoagulable specimens at three dilutions of rTF compared to the min_1 values of the ratio of the same dilution of a normal plasma. All of the ratios of the hypocoagulable plasmas for all three dilutions are less than the normal response (values of <1). As the dilution increase, i.e. less tissue factor is provided, the difference in the ratios increases.

Figure 9 illustrates ratios of the min_1 values for hypercoagulable specimens at three dilutions of rTF and 10nM thrombomodulin compared to min_1 values of the ratio for the same conditions of a normal plasma. All of the ratios of the hypercoagulable

plasmas for all three dilutions are greater than the normal response (values of >1). As the dilution increase, i.e. less tissue factor is provided, the difference in the ratios increases.

Figure 10 illustrates the effects on min_1 values of varying tissue factor and thrombomodulin concentrations on results for hypercoagulable, hypocoagulable and normal plasmas. The data indicate that an optimal concentration can be defined to facilitate differentiation between normal, hypercoagulable and hypocoagulable plasmas. Additionally, other concentrations of tissue factor and thrombomodulin facilitate improvements in sensitivity and specificity for a particular condition at the expense of the sensitivity and specificity of another type of condition.

Tables 2 and 3 summarize the results of measuring the kinetic parameters, min 1 and min 2 with a series of defined patient plasmas. The concentration of TF was 10 pM and TM was adjusted to 10 nM. The phospholipid concentration was kept constant at 150 micromolar. The data shows that the reagent in the presence of TM is able to differentiate hyper and hypocoagulable plasmas with a single reagent formulation. Additionally, the data indicates that TM is not essential to obtain discrimination between the hypocoagulable and a normal standard plasma pool. Data are calculated as ratios to a normal pool with and without thrombomodulin. Ratios of the min2 parameter were higher than the corresponding min1 values for the hypercoagulable plasmas.

Tables 2 and 3 illustrate the behavior of defined plasmas in the presence and absence of thrombomodulin as determined by the kinetic endpoints min_1 and min_2.

Table 2

Plasma Type	Min _1 values with no TM	Min _1 values with 10 nM TM	Min_1 Ratio Specimen without TM/Normal plasma without TM	Min_1 Ratio Specimen with 10 nM TM/Normal plasma with 10 nM TM
Normal Plasma	101	68		
PC Deficient	110	105	1.09	1.54

Lupus	116	79	1.15	1.16
FV Leiden	95	77	0.94	1.13
FV Leiden & PT 20210	260	248	2.57	3.64
FIX Deficient	71	40	0.70	0.59
FVIII Deficient	84	46	0.83	0.68

Table 3

Plasma Type	Min_2 values with no TM	Min_2 values with 10 nM TM	Min_2 Ratio Specimen without TM/Normal plasma without TM	Min_2 Ratio Specimen with 10 nM TM/Normal plasma with 10 nM TM
Normal Plasma	34.8	11.9		
PC Deficient	36.4	27.6	1.05	2.32
Lupus	47.4	23.6	1.36	1.98
FV Leiden	32.6	20.3	0.94	1.71
FV Leiden & PT 20210	181	165	5.2	13.9
FIX Deficient	21	9	0.60	0.76
FVIII Deficient	16.4	6.4	0.47	0.54

Example 1:

The assay was conducted by adding 50uL of plasma to 50uL of the activator and then adding 50uL of the start reagent. A normal sample, a hypocoagulable sample (Factor VIII deficient plasma) and a hypercoagulable plasma (protein S deficient plasma) were evaluated at various dilutions of the activator. The activator was a commercially available thromboplastin (Thromborel R, Behring Diagnostics) diluted with a buffer at two dilutions, a 1:100 and 1:50000 of its original concentration. The start reagent consisted of 0.25 M Calcium Chloride. The assay was conducted at 37 C and the reaction was monitored at 580nm for 300 seconds. Endpoints were calculated for time and rate indices of clot formation. Ratios of the endpoints were compared to other dilutions and other samples as follows:

$$\text{Ratio} = \frac{\text{endpoint of reagent diln (x) for Specimen/endpoint of reagent diln (y) for specimen}}{\text{endpoint of reagent diln (x) for npp / endpoint of reagent diln (y) for npp}}$$

Where x is a 1:100 dilution and y is a series of dilutions

As the dilution of the reagent become greater (y becomes larger) the results for the two abnormal plasmas (the aforementioned hypercoagulabe and hypocoagulable plasmas) tested began to deviate from the calculated endpoints or ratios of the normal plasma. The results can be expressed as the magnitude of deviation at a given dilution or as the dilution required to deviate from ideal (normal value or normal range). Figure 7 illustrates that the hypercoagulable and hypocoagulable results deviate in opposite directions indicating the ability to differentiate between the two conditions.

Example II

The assay was conducted by adding 50uL of plasma to 50uL of the activator and then adding 50uL of the start reagent. A set of normal samples, a series of samples from hypocoagulable individuals and a series of plasmas from hypercoagulable individuals were evaluated at various dilutions of the activator. The activator was a preparation of TF reconstituted with phospholipids to between 20 to 3.3 pM (1:20,000 to 1:120,000 dilution) and phospholipid prepared by extrusion with and without TM. The start reagent consisted of 0.025 M Calcium Chloride. The assay was conducted at 37 C and the reaction was monitored at 580nm for 300 seconds. The value of the minimum of the 1^{st} derivative and the value of the minimum of the 2^{nd} derivative were calculated for all samples. Ratios of the endpoints were compared to other dilutions and other samples as follows:

Option 1 - Endpoint(s)

Option 2 - Ratio at different dilutions (ratio 1)

$$\frac{\text{Endpoint (z) for dilution (x)}}{\text{Endpoint (z) for dilution (y)}}$$

Option 3 - Ratio of dilutions compared to normal (ratio 2)

$$\frac{\text{Ratio 1 for patient sample}}{\text{Ratio 1 for normal plasma}}$$

Option 4 - Ratio for different reagent formulations

Ratio 2 with formulation (a)

Ratio 2 with formulation (b)

Option 5 - Ratio of specimen to normal at a given dilution

Endpoint (z) at dilution x for a specimen

Endpoint (z) at dilution x for a normal plasma

Figures 8 and 9 illustrate the differentiation for hypercoagulable and hypercoagulable specimens when compared to normal. Tables 2 and 3 illustrate the behavior of defined plasmas in the presence and absence of thrombomodulin as determined by the kinetic endpoints min_1 and min_2. Figure 10 demonstrates the effect of varying tissue factor and thrombomodulin on the results from hypercoagulable, hypercoagulable and normal plasmas. The data indicate that variations in the concentrations facilitate improvements in sensitivity and specificity for a condition at the expense of the sensitivity and specificity of another type of condition.

In one preferred embodiment, the TF is added to the sample at a concentration of about less than or equal to 10 picomolar and the phospholipid concentration of between 10 to 300 μM. The TF can be added to the sample at a concentration of 3 to 10 picomolar and the phospholipid vesicles can be added at 100 to 150 micromolar. Preferably thrombomodulin is added at 0 to 30 nanomolar and most preferably at a concentration of 5 to 15 nanomolar. Calcium Chloride is most preferably added at a concentration of about 25 mM. All of the reagent component concentrations described are further diluted 1:3 in the plasma/buffer matrix in the cuvette.

One or more parts or endpoints of the time dependent measurement profile obtained by monitoring fibrin polymerization in the test sample can be compared to the same parts or endpoints of a time dependent measurement profile obtained by monitoring fibrin polymerization in the test sample at a different coagulation activator concentration and/or to the same parts or endpoints for a known (e.g.

normal) test sample. The part of the profile can be one or more of initiation of clot formation, overall change in profile, slope of profile after initiation of clot formation, and acceleration at the time of clot initiation. Also, if at least two time-dependent fibrin polymerization profiles are obtained, an additional profile can be obtained for a known sample from computer memory or by adding the activator at at least one concentration to a known sample and monitoring the formation of fibrin polymerization over time. The parameter from each time-dependent fibrin polymerization profile having varying activator concentrations can be determined and a concentration at which the at least one parameter of said sample being tested deviates from normal can be determined. The point of deviation is indicative of the hypercoagulable or hypocoagulable state. The part of the profile is preferably a time index of the minimum of the first derivative, the value of the minimum of the first derivative, the time index for the minimum of the second derivative, the value for the minimum of the second derivative, the time index of the maximum of the second derivative, the value of the maximum of the second derivative, or the overall magnitude of change. More preferably, the part is rate or acceleration of fibrin polymerization, wherein the rate or acceleration is compared to rate or acceleration at the same activator concentration for the known sample.

Though endpoints can be directly compared as noted above, a difference or ratio of said parameters for said test sample and said normal sample can instead be determined. If the parameter is clot time, a ratio of clot times at different activator concentrations can be determined. A ratio of other parameters, rate of clot formation, maximum acceleration of clot formation, turbidity at a predetermined time period, and total change in turbidity can also be determined in order to measure defects in the thrombin propagation and/or amplification phases. Also, a ratio can be taken of the at least one parameter for said test sample to the same parameter for a normal sample. And, the ratio can be determined for multiple concentrations of activator to better characterize the hypo- or hyper-coagulability. For example, the concentration at which said ratio

(test sample/known sample) departs from 1 (or a range around 1) can show the abnormal coagulability.

Other ratios aid determination of the hemostatic potential (e.g. the hypocoagulability, stasis, or hypercoagulability; or the bleeding or thrombotic tendency of the patient). For example, a first ratio can be calculated for the at least one parameter at two different concentrations of the activator. A second ratio can be calculated of said first ratio at the two different activator concentrations relative to a first ratio calculated for a known sample at two different activator concentrations. A third ratio can be calculated of the second ratio at a first reagent formulation and the second ratio at a second reagent formulation. Though the second reagent can vary in a number of ways from the first, in one embodiment the first reagent formulation can comprise a coagulation activator and the second reagent formulation can comprise a coagulation activator and an activator of an anticoagulant pathway. A fourth ratio could be calculated of the second ratio calculated for one endpoint relative to the second ratio calculated for a different endpoint. Significant information can be obtained by changing the reagent formulation and comparing the same endpoints, or by maintaining the reagent formulation (though possibly at a different concentration) and comparing different endpoints (or both endpoint and reagent formulation and/or concentration can be altered).

An activator of one or more anticoagulant pathways can be added along with the coagulation activator. Such an additional activator can be any activator of an anticoagulant pathway, such as the protein C pathway. Thrombomodulin is one example, which can be added in the form of purified human thrombomodulin, purified non-human mammalian thrombomodulin, soluble or membrane associated thrombomodulin, native thrombomodulin or reconstituted with phospholipids, partially or fully glycolsylated thrombomodulin, or fully deglycosylated thrombomodulin, with added heparin-like molecules. The coagulation activator can be any suitable activator including recombinant or purified tissue factor, truncated tissue factor, or cells expressing tissue factor on their surface. If vesicles or liposomes are added, they can be in the form

of platelets, cellular debris, phospholipids or platelet microparticles. A metal salt if added can be a halide of magnesium, calcium or manganese, or other divalent metal salt. Buffers and stabilizers could also be added if desired.

A reagent or kit for assessing hemostatic potential should have a coagulation activator. Additional components of the reagent or kit could include the above-mentioned vesicles, metal salt or ions, and anticoagulant pathway activator, if desired. In the kit, the components could all be provided in separate containers, or mixed together in any combinations in one or more containers. If phospholipid vesicles are added, they can be any suitable phospholipid or combination of phospholipids including one or more of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, which can be provided at a ratio of approximately 5 to 30 mole percent phosphatidylethanolamine, 1 to 10 percent phosphatidylserine and the remainder phosphatidylcholine. These vesicles can be prepared in a variety of ways to yield liposomes of various sizes. Phospholipids can be provided at a concentration that is not rate limiting, e.g. at a concentration of from 10 to 300 micromolar, and preferably in the range of from 50 to 200 micromolar. Tissue factor can be provided at a concentration of 10 picomolar or less, 8 picomolar or less, or preferably 6 picomolar or less. The concentration could be 3 picomolar or less, though whatever concentration of tissue factor, it should allow for hemostatic potential assessment as set forth herein. If it is desired to add thrombomodulin, it can be provided at a concentration of 30 nanomolars or less, preferably in a range of from 5 to 20 nanomolar. If a metal salt is to be added, it can be provided in a reagent or kit at a concentration of from 5 to 50 mM, preferably from 15 to 35 mM.

Variations to the above described method, kit and reagent are possible, and the embodiments disclosed herein should be considered illustrative and not limiting.

We claim:

1. A method for determining if a patient is hypercoagulable, hypocoagulable or normal, comprising:

a) providing a test sample from the patient;

b) initiating coagulation in the sample in the presence of an activator, which is added to the sample in an amount which will result in intrinsic tenase-dependent fibrin polymerization;

c) monitoring formation of said intrinsic tenase-dependent fibrin polymerization over time so as to derive a time-dependent profile, wherein results of said fibrin polymerization monitoring determine whether said patient is hypercoagulable, normal or hypocoagulable.

2. The method according to claim 1, wherein all or part of said time-dependent profile is compared to all or part of a time-dependent profile for a known sample.

3. The method according to claim 2, wherein part of said profile is compared, said part of said profile including one or more of initiation of clot formation, overall change in profile, slope of profile after initiation of clot formation, and acceleration at the time of clot initiation.

4. The method according to claim 2, wherein at least two time-dependent fibrin polymerization profiles are obtained, an additional profile being obtained for a known sample from computer memory or by adding said activator at at least one concentration to a known sample and monitoring the formation of fibrin polymerization over time.

5. The method according to claim 4, wherein at least two time-dependent fibrin polymerization profiles are obtained, one profile for said test sample at a first activator concentration, and at least one additional profile for said test sample at a second activator concentration and/or one or more profiles for a known sample at one or more activator concentrations.

6. The method according to claim 1, wherein the activator comprises tissue factor.

7. The method according to claim 4, wherein at least one parameter from each time-dependent fibrin polymerization profile having varying activator concentrations is determined and a concentration at which the at least one parameter of said sample being tested deviates from normal is determined.

8. The method according to claim 7, wherein said at least one parameter is selected from time index and value of the minimum of the first derivative, the time index and value for the minimum and maximum of the second derivative and the overall magnitude of change.

9. The method according to claim 5, wherein part of each fibrin polymerization profile is compared to a same part of a profile for a known sample.

10. The method according to claim 9, wherein said part is one or more of a time index of the minimum of the first derivative, the value of the minimum of the first derivative, the time index for the minimum of the second derivative, the value for the minimum of the second derivative, the time index of the maximum of the second derivative, the value of the maximum of the second derivative, and the overall magnitude of change.

11. The method according to claim 9, wherein said part is rate or acceleration of fibrin polymerization, wherein said rate or acceleration is compared to rate or acceleration at the same activator concentration for said known sample.

12. The method according to claim 9, wherein a difference or ratio of said parameters for said test sample and said normal sample are determined.

13. The method according to claim 12, wherein said parameter is clot time and a ratio of clot times at different activator concentrations is determined.

14. The method according to claim 1, wherein one or more parameters of said time-dependent fibrin polymerization profile are compared to the same one or more parameters for a normal sample, in order to determine whether said patient is hypercoagulable, normal or hypocoagulable.

15. The method according to claim 7, wherein said at least one parameter includes at least one of time of initiation of clot formation, rate of clot formation, maximum acceleration of clot formation, turbidity at a predetermined time period, and total change in turbidity.

16. The method according to claim 15 wherein said one or more parameters are measures of defects in the thrombin propagation and/or amplification phases.

17. The method according to claim 15, wherein a ratio of said at least one parameter for said test sample to the same parameter for a normal sample is determined.

18. The method according to claim 17, wherein said ratio is determined for multiple concentrations of activator.

19. The method according to claim 18, wherein a concentration at which said ratio departs from 1 is determined.

20. The method according to claim 1, wherein an activator of one or more anticoagulant pathways is added.

21. The method according to claim 20, wherein an activator of protein C is added.

22. The method according to claim 21, wherein the protein C activator is thrombomodulin.

23. The method according to claim 22, wherein a fibrin polymerization profile is obtained with and without said thrombomodulin.

24. The method according to claim 1, wherein multiple concentrations of said activator are used for providing corresponding multiple time-dependent measurement profiles, and multiple concentrations of activator of a known sample are used for providing corresponding multiple time-dependent known sample measurement profiles, and ratios of one or more parameters of the measurement profiles of the known and test sample are compared.

25. The method according to claim 24, wherein the one or more parameters at the one or more concentrations of said activator can be compared in the presence or absence of a modulator of one or more anticoagulant pathways.

26. The method according to claim 1, wherein one or more parameters at multiple concentrations of said activator are determined and results are compared.

27. The method according to claim 24, wherein any concentration of said activator can be compared in the presence or absence of a modulator of one or more anticoagulant pathways.

28. The method according to claim 27, wherein the activator is tissue factor and the modulator is thrombomodulin.

29. The method according to claim 1, wherein the activator comprises tissue factor and phospholipids.

30. The method according to claim 1, wherein a metal salt is added as part of the activator or separately therefrom, which metal salt dissociates into a metal divalent cation when added to the test sample.

31. The method according to claim 30, wherein the divalent metal cation is magnesium, calcium or manganese.

32. The method of claim 30, wherein the metal salt is a halide of magnesium, calcium or manganese.

33. The method of claim 1, wherein the activator comprises purified or recombinant tissue factor.

34. The method of claim 33, wherein the activator comprises homogenized cerebral tissue.

35. The method of claim 1, further comprising adding phopholipids together with or separately from the activator.

36. The method of claim 1, further comprising adding buffers and/or stabilizers to the test sample.

37. The method of claim 1, wherein the test sample is a patient plasma sample.

38. The method of claim 2, wherein the known sample is a normal sample.

39. The method of claim 1, wherein the time dependent measurement profile is an optical absorbance or transmittance profile provided on an automated analyzer.

40. The method of claim 39, wherein a light beam having a wavelength in the visible spectrum is directed through a container holding the test sample and activator, and light absorbed or transmitted is monitored to form the time dependent measurement profile.

41. The method of claim 1, wherein the activator comprises tissue factor sufficiently diluted so as to allow determination of any of hypercoagulable, normal or hypcoagulable depending upon the condition of the patient.

42. The method of claim 1, wherein a part of the time dependent measurement profile other than clot time is compared to the same part of a time dependent measurement profile for a known sample.

43. The method of claim 1, wherein defects in formation of intrinsic tenase complex are detected.

44. The method of claim 1, wherein one or more endpoints from the time-dependent measurement profile are calculated, the endpoints selected from the time of clot initiation and the rate of polymerization.

45. The method of claim 44, wherein at least one parameter selected from the first derivative of the time dependent measurement profile, the second derivative of the time dependent measurement profile, the minimum of the first and/or second derivative, or the maximum of the first and/or second derivative are calculated with respect to value and/or the time associated time index.

46. The method of claim 45, wherein the at least one parameter is compared to the same at least one parameter for a known sample.

47. The method of claim 45, wherein a first ratio is calculated for the at least one parameter at two different concentrations of the activator.

48. The method of claim 47, wherein a second ratio is calculated of said first ratio at the two different activator concentrations relative to a first ratio calculated for a known sample at two different activator concentrations.

49. The method of claim 48, wherein a third ratio is calculated of said second ratio at a first reagent formulation and said second ratio at a second reagent formulation.

50. The method of claim 49, wherein the first reagent formulation comprises a coagulation activator and the second reagent formulation comprises a coagulation activator and an activator of an anticoagulant pathway.

51. The method of claim 50, wherein the first reagent comprises tissue factor and the second reagent comprises tissue factor and thrombomodulin.

52. The method of claim 48, wherein a fourth ratio is calculated of said second ratio calculated for one endpoint relative to said second ratio calculated for a different endpoint.

53. The method of claim 52, wherein one of the endpoints is clot time and the other is the minimum of the first derivative.

54. The method of claim 1, wherein sample is whole blood or platelet rich plasma.

55. The method of claim 1, further comprising adding vesicles to the test sample.

56. The method of claim 55, wherein the vesicles comprise platelets, cellular debris, phospholipid vesicles or platelet microparticles.

57. The method of claim 1, further comprising adding a protein C activator to the test sample.

58. The method according to claim 57, wherein the protein C activator is purified human thrombomodulin, purified non-human mammalian thrombomodulin, soluble or membrane associated thrombomodulin, native thrombomodulin or thrombomodulin reconstituted with phospholipids, partially or fully glycolsylated thrombomodulin or fully deglycosylated thrombomodulin.

59. The method of claim 1, wherein the activator comprises recombinant or purified tissue factor, truncated tissue factor, or cells expressing tissue factor on their surface.

60. A method for assessing the coagulation system in a test sample, comprising:
providing a sample to be tested;
adding an activator to said sample to trigger a thrombin explosion dependent on propagation phase and amplification loops and subject to one or more anticoagulant pathways;
measuring the polymerization of fibrin due to said thrombin explosion; and
assessing the coagulation system in said test sample based on said measured fibrin polymerization.

61. The method of claim 60, further comprising adding vesicles to the test sample.

62. The method of claim 61, wherein the vesicles comprise platelets, cellular debris, phospholipid vesicles or platelet microparticles.

63. The method of claim 60, wherein an activator of protein C is added to cause the fibrin polymerization to be sensitive to the protein C pathway.

64. The method according to claim 63, wherein the protein C activator is purified human thrombomodulin, purified non-human mammalian thrombomodulin, soluble or membrane associated thrombomodulin, native thrombomodulin or thrombomodulin reconstituted with phospholipids, partially or fully glycolsylated thrombomodulin or fully deglycosylated thrombomodulin.

65.The method of claim 60, wherein the activator comprises recombinant or purified tissue factor, truncated tissue factor, or cells expressing tissue factor on their surface.

66. The method of claim 60, wherein the fibrin polymerization is monitored over time to provide a time-dependent measurement profile.

67. The method of claim 66, wherein an endpoint is extracted from the time-dependent measurement profile.

68. The method of claim 67, wherein the endpoint is normalized by using a model.

69. The method of claim 68, wherein the model is a ratio or difference of the endpoint compared to an endpoint from a time-dependent measurement profile for a known sample.

70. The method of claim 69, wherein the endpoint is initiation of clot formation, overall change in the profile, or slope of the profile after initiation of clot formation.

71. The method according to claim 66, wherein at least two time-dependent fibrin polymerization profiles are obtained, an additional profile being obtained for a known sample from computer memory or by adding said activator at at least one concentration to a known sample and monitoring the formation of fibrin polymerization over time.

72. The method according to claim 71, wherein at least one parameter from each time-dependent fibrin polymerization profile having varying activator concentrations is determined and a concentration at which the at least one parameter of said sample being tested deviates from normal is determined.

73. The method according to claim 67, wherein the endpoint is time index or value of the minimum of the first derivative, the time index or value for the minimum or maximum of the second derivative, or the overall magnitude of change.

74. The method according to claim 66, wherein the rate or acceleration of fibrin polymerization is determined from the time-dependent measurement profile, wherein said rate or acceleration is compared to rate or acceleration at the same activator concentration for a known sample and/or the rate or acceleration of the test sample at a different activator concentration.

75. The method of claim 63, wherein a fibrin polymerization profile is obtained with and without a protein C activator.

76. The method of claim 75, wherein a fibrin polymerization profile is obtained at multiple concentrations of said activator which triggers thrombin explosion.

77. The method of claim 76, wherein a fibrin polymerization profile is obtained at multiple concentrations for a known sample.

78. A method for detecting defects in the propagation and/or amplification phase in the coagulation system of a test sample, comprising:

providing a sample to be tested;

adding an activator capable of triggering a thrombin explosion that is dependent on the propagation phase and/or amplification loops of the coagulation system in the test sample;

measuring fibrin polymerization; and

detecting defects of regulation or modulation in the propagation phase and/or amplification loops in the coagulation system of the test sample based on the measured fibrin polymerization.

79. The method according to claim 78, wherein all or part of said time-dependent profile is compared to all or part of a time-dependent profile for a known sample.

80. The method according to claim 79, wherein part of said profile is compared, said part of said profile including one or more of initiation of clot formation, overall change in profile, slope of profile after initiation of clot formation and acceleration at the time of clot initiation.

81. The method according to claim 79, wherein at least two time-dependent fibrin polymerization profiles are obtained, an additional profile being obtained for a known sample from

computer memory or by adding said activator at at least one concentration to a known sample and monitoring the formation of fibrin polymerization over time.

82. The method according to claim 81, wherein at least two time-dependent fibrin polymerization profiles are obtained, one profile for said test sample at a first activator concentration, and at least one additional profile for said test sample at a second activator concentration and/or one or more profiles for a known sample at one or more activator concentrations.

83. The method according to claim 78, wherein the activator comprises tissue factor.

84. The method according to claim 81, wherein at least one parameter from each time-dependent fibrin polymerization profile having varying activator concentrations is determined and a concentration at which the at least one parameter of said sample being tested deviates from normal is determined.

85. The method according to claim 84, wherein said at least one parameter is time index and value of the minimum of the first derivative, the time index and value for the minimum and maximum of the second derivative and the overall magnitude of change.

86. The method according to claim 82, wherein part of each fibrin polymerization profile is compared to a same part of a profile for a known sample.

87. The method according to claim 86, wherein said part is one or more of a time index of the minimum of the first derivative, the value of the minimum of the first derivative, the time index for the

minimum of the second derivative, the value for the minimum of the second derivative, the time index of the maximum of the second derivative, the value of the maximum of the second derivative, and the overall magnitude of change.

88. The method according to claim 88, wherein said part is rate or acceleration of fibrin polymerization, wherein said rate or acceleration is compared to rate or acceleration at the same activator concentration for said known sample.

89. The method according to claim 88, wherein a difference or ratio of said parameters for said test sample and said normal sample are determined.

90. The method according to claim 89, wherein said parameter is clot time and a ratio of clot times at different activator concentrations is determined.

91. The method according to claim 78, wherein one or more parameters of said time-dependent fibrin polymerization profile are compared to the same one or more parameters for a normal sample, in order to determine whether said patient is hypercoagulable, normal or hypocoagulable.

92. The method according to claim 84, wherein said at least one parameter includes at least one of time of initiation of clot formation, rate of clot formation, maximum acceleration of clot formation, turbidity at a predetermined time period, and total change in turbidity.

93. The method according to claim 92 wherein said one or more parameters are measures of defects in the thrombin propagation and/or amplification phases.

94. The method according to claim 92, wherein a ratio of said at least one parameter for said test sample to the same parameter for a normal sample is determined.

95. The method according to claim 94, wherein said ratio is determined for multiple concentrations of activator.

96. The method according to claim 95, wherein a concentration at which said ratio departs from 1, or a range around 1, is determined.

97. The method according to claim 78, wherein an activator of one or more anticoagulant pathways is added.

98. The method according to claim 97, wherein an activator of protein C is added.

99. The method according to claim 98, wherein the protein C activator is thrombomodulin.

100. The method according to claim 99, wherein a fibrin polymerization profile is obtained with and without said thrombomodulin.

101. The method according to claim 78, wherein multiple concentrations of said activator are used for providing corresponding multiple time-dependent measurement profiles, and multiple concentrations of activator of a known sample are used for providing corresponding multiple time-dependent known sample measurement profiles, and ratios of one or more parameters of the measurement profiles of the known and test sample are compared.

102. The method according to claim 101, wherein the one or more parameters at the one or more concentrations of said activator can be compared in the presence or absence of a modulator of one or more anticoagulant pathways.

103. The method according to claim 78, wherein one or more parameters at multiple concentrations of said activator are determined and results are compared.

104. The method according to claim 101, wherein any concentration of said activator can be compared in the presence or absence of a modulator of one or more anticoagulant pathways.

105. The method according to claim 104, wherein the activator is tissue factor and the modulator is thrombomodulin.

106. The method according to claim 78, wherein the activator comprises tissue factor and phospholipids.

107. The method according to claim 78, wherein a metal salt is added as part of the activator or separately therefrom, which metal salt dissociates into a metal divalent cation when added to the test sample.

108. The method according to claim 107, wherein the divalent metal cation is magnesium, calcium or manganese.

109. The method of claim 107, wherein the metal salt is a halide of magnesium, calcium or manganese.

110. The method of claim 78, wherein the activator comprises purified or recombinant tissue factor.

111. The method of claim 110, wherein the activator comprises homogenized brain tissue.

112. The method of claim 78, further comprising adding phopholipids together with or separately from the activator.

113. The method of claim 78, further comprising adding buffers and/or stabilizers to the test sample.

114. The method of claim 78, wherein the test sample is a patient plasma sample.

115. The method of claim 79, wherein the known sample is a normal sample.

116. The method of claim 78, wherein the time dependent measurement profile is an optical absorbance or transmittance profile provided on an automated analyzer.

117. The method of claim 116, wherein a light beam having a wavelength in the visible spectrum is directed through a container holding the test sample and activator, and light absorbed or transmitted is monitored to form the time dependent measurement profile.

118. The method of claim 78, wherein the activator comprises tissue factor sufficiently diluted so as to allow determination of any of hypercoagulable, normal or hypcoagulable depending upon the condition of the patient.

119. The method of claim 78, wherein a part of the time dependent measurement profile other than clot time is compared to the same part of a time dependent measurement profile for a known sample.

120. The method of claim 78, wherein defects in formation of intrinsic tenase complex are detected.

121. The method of claim 78, wherein one or more endpoints from the time-dependent measurement profile are calculated, the endpoints selected from the time of clot initiation and the rate of polymerization.

122. The method of claim 121, wherein at least one parameter selected from the first derivative of the time dependent measurement profile, the second derivative of the time dependent measurement profile, the minimum of the first and/or second derivative, or the maximum of the first and/or second derivative are calculated with respect to value and/or the time associated time index.

123. The method of claim 122, wherein the at least one parameter is compared to the same at least one parameter for a known sample.

124. The method of claim 122, wherein a first ratio is calculated for the at least one parameter at two different concentrations of the activator.

125. The method of claim 124, wherein a second ratio is calculated of said first ratio at the two different activator concentrations relative to a first ratio calculated for a known sample at two different activator concentrations.

126. The method of claim 125, wherein a third ratio is calculated of said second ratio at a first reagent formulation and said second ratio at a second reagent formulation.

127. The method of claim 126, wherein the first reagent formulation comprises a coagulation activator and the second reagent formulation comprises a coagulation activator and an activator of an anticoagulant pathway.

128. The method of claim 127, wherein the first reagent comprises tissue factor and the second reagent comprises tissue factor and thrombomodulin.

129. The method of claim 125, wherein a fourth ratio is calculated of said second ratio calculated for one endpoint relative to said second ratio calculated for a different endpoint.

130. The method of claim 129, wherein one of the endpoints is clot time and the other is the minimum of the first derivative.

131. The method of claim 78, wherein sample is whole blood or platelet rich plasma.

132. The method of claim 78, further comprising adding vesicles to the test sample.

133. The method of claim 132, wherein the vesicles comprise platelets, cellular debris, lipids or platelet microparticles.

134. The method of claim 78, further comprising adding a protein C activator to the test sample.

135. The method according to claim 134, wherein the protein C activator is purified human thrombomodulin, purified non-human mammalian thrombomodulin, soluble or membrane associated thrombomodulin, native thrombomodulin or thrombomodulin reconstituted with phospholipids, partially or fully glycolsylated thrombomodulin or fully deglycosylated thrombomodulin.

136. The method of claim 78, wherein the activator comprises recombinant or purified tissue factor, truncated tissue factor, or cells expressing tissue factor on their surface.

137. A method for determining whether a patient is hypercoagulable, normal or hypocoagulable, comprising:
providing a sample to be tested from a patient;
adding less than 11 picomolar concentration of tissue factor to said sample, said tissue factor generating intrinsic dependent fibrin polymerization in said sample;
measuring formation of the fibrin polymerization; and
determining whether said patient is hypercoagulable, normal or hypocoagulable based on said measured fibrin polymerization.

138. The method according to claim 137, wherein said fibrin polymerization is measured over time so as to derive a time-dependent fibrin polymerization profile.

139. The method according to claim 138, wherein one or more parameters of said fibrin polymerization profile are compared to the same parameters of a fibrin polymerization profile for a normal sample or for the same test sample where the activator or the activator concentration is changed.

140. The method according to claim 139, wherein said one or more parameters do not include clot time.

141. The method of claim 139, wherein the one or more parameters are determined or calculated based on information in the time dependent measurement profiles which are after initiation of clot formation.

142. The method according to claim 141, wherein said one or more parameters include the rate of fibrin polymerization.

143. The method according to claim 137, wherein said sample comprises endogenous or exogenous fibrinogen.

144. The method according to claim 143, wherein the measurement of fibrin polymerization is performed in the absence of a chromogenic substrate in the test sample.

145. The method according to claim 137, wherein the test sample is a non-diluted native plasma sample and the activator added thereto comprises tissue factor.

146. The method according to claim 145, further comprising adding phosphatidylcholine, phosphatidylethanolamine and/or phosphatidylserine as part of the activator or separately therefrom.

147. The method according to claim 137, wherein at least a portion of said time-dependent profile or a value derived therefrom is compared to the same portion or value for a known sample.

148. The method according to claim 147, wherein part of said profile is compared, said part of said profile including one or more of initiation of clot formation, overall change in profile, and slope of profile after initiation of clot formation.

149. The method according to claim 147, wherein at least two time-dependent fibrin polymerization profiles are obtained, an additional profile being obtained for a known sample from computer memory or by adding said activator at at least one concentration to a known sample and monitoring the formation of fibrin polymerization over time.

150. The method according to claim 149, wherein at least two time-dependent fibrin polymerization profiles are obtained, one profile for said test sample at a first activator concentration, and at least one additional profile for said test sample at a second activator concentration and/or one or more profiles for a known sample at one or more activator concentrations.

151. The method according to claim 137, wherein the activator comprises tissue factor.

152. The method according to claim 149, wherein at least one parameter from each time-dependent fibrin polymerization profile at a different activator concentration is determined and a concentration at which the at least one parameter of said sample being tested deviates from normal, or a range around normal, is determined.

153. The method according to claim 152, wherein said parameter is one or more of a time index of the minimum of the first derivative, the value of the minimum of the first derivative, the time index for the minimum of the second derivative, the value for the minimum of the second derivative, the time index of the maximum of the second derivative, the value of the maximum of the second derivative, and the overall magnitude of change.

154. The method according to claim 152, wherein said parameter is rate or acceleration of fibrin polymerization, wherein said rate or acceleration is compared to rate or acceleration at the same activator concentration for said known sample.

155. The method according to claim 152, wherein a difference or ratio of said parameters for said test sample and said known sample are determined.

156. The method according to claim 152 wherein said at least one parameter is a measure of defects in the thrombin propagation and amplification phases.

157. The method according to claim 155, wherein said ratio is determined for multiple concentrations of activator.

158. The method according to claim 155, wherein a concentration at which said ratio departs from 1, or a range around 1, is determined.

159. The method according to claim 137, further comprising adding an activator of one or more anticoagulant pathways.

160. The method according to claim 159, wherein an activator of protein C is added.

161. The method according to claim 160, wherein the protein C activator is thrombomodulin.

162. The method according to claim 161, wherein a fibrin polymerization profile is obtained with and without said thrombomodulin.

163. The method according to claim 137, wherein multiple concentrations of said activator are used for providing corresponding multiple time-dependent measurement profiles, and multiple concentrations of activator of a known sample are used for providing corresponding multiple time-dependent known sample measurement profiles, and ratios of one or more parameters of the measurement profiles of the known and test sample are compared.

164. The method according to claim 137, wherein any concentration of said activator can be compared in the presence or absence of a modulator of one or more anticoagulant pathways.

165. The method according to claim 137, wherein a metal salt is added as part of the activator or separately therefrom, which metal salt dissociates into a metal divalent cation when added to the test sample.

166. The method according to claim 165, wherein the divalent metal cation is magnesium, calcium or manganese.

167. The method of claim 165, wherein the metal salt is a halide of magnesium, calcium or manganese.

168. The method of claim 137, wherein the activator comprises purified or recombinant tissue factor.

169. The method of claim 168, wherein the activator comprises homogenized brain tissue.

170. The method of claim 137, further comprising adding phospholipids together with or separately from the activator.

171. The method of claim 137, further comprising adding buffers and/or stabilizers to the test sample.

172. The method of claim 137, wherein the time dependent measurement profile is an optical absorbance or transmittance profile provided on an automated analyzer.

173. The method of claim 137, wherein the activator comprises tissue factor sufficiently diluted so as to allow determination of any of hypercoagulable, normal or hypcoagulable depending upon the condition of the patient.

174. The method of claim 137, wherein a part of the time dependent measurement profile other than clot time is compared to the same part of a time dependent measurement profile for a known sample.

175. The method of claim 137, wherein defects in formation of intrinsic tenase complex are detected.

176. The method of claim 137, wherein a first ratio is calculated for the at least one parameter at two different concentrations of the activator.

177. The method of claim 176, wherein a second ratio is calculated of said first ratio at the two different activator concentrations relative to a first ratio calculated for a known sample at two different activator concentrations.

178. The method of claim 177, wherein a third ratio is calculated of said second ratio at a first reagent formulation and said second ratio at a second reagent formulation.

179. The method of claim 178, wherein the first reagent formulation comprises a coagulation activator and the second reagent formulation comprises a coagulation activator and an activator of an anticoagulant pathway.

180. The method of claim 179, wherein the first reagent comprises tissue factor and the second reagent comprises tissue factor and thrombomodulin.

181. The method of claim 177, wherein a fourth ratio is calculated of said second ratio calculated for one endpoint relative to said second ratio calculated for a different endpoint.

182. The method of claim 181, wherein one of the endpoints is clot time and the other is the minimum of the first derivative.

183. The method of claim 137, further comprising adding vesicles to the test sample.

184. The method of claim 182, wherein the vesicles comprise platelets, cellular debris, phospholipid vericles or platelet microparticles.

185. The method of claim 137, further comprising adding a protein C activator to the test sample.

186. The method according to claim 185, wherein the protein C activator is purified human thrombomodulin, purified non-human mammalian thrombomodulin, soluble or membrane associated thrombomodulin, native thrombomodulin or thrombomodulin reconstituted with phospholipids, partially or fully glycolsylated thrombomodulin or fully deglycosylated thrombomodulin.

187. The method of claim 137, wherein the activator comprises recombinant or purified tissue factor, truncated tissue factor, or cells expressing tissue factor on their surface.

188. A method for monitoring an antithrombotic or procoagulant pharmaceutical therapy, comprising:

providing a first test sample from a patient;

adding an activator to said test sample in order to trigger a thrombin explosion dependent upon the propagation phase and amplification loops of the coagulation system in the test sample;

measuring fibrin polymerization due at least in part to said thrombin explosion;

determining whether the patient is hypocoagulable, normal or hypercoagulable, or providing a baseline;

if the patient is hypercoagulable or hypocoagulable, adminstering one or more antithrombotic or procoagulant pharmaceuticals to said patient;

providing at least one additional sample from said patient at a time after administration of the pharmaceutical;

adding said activator to said at least one additional sample in order to trigger a thrombin explosion dependent upon the propagation phase and amplification loops of the coagulation system in the test sample;

measuring fibrin polymerization in said second sample due at least in part to said thrombin explosion;

determining whether the second patient sample is hypocoagulable, normal or hypercoagulable, or determining a change from baseline; and

determining the effectiveness of the pharmaceutical therapy based on any changes in the hypocoagulability or hypercoagulability from the first test sample, or any changes from baseline.

189. The method of claim 188, further comprising adding vesicles to the test sample.

190. The method of claim 189, wherein the vesicles comprise platelets, cellular debris, phospholipid vesicles or platelet microparticles.

191. The method of claim 188, wherein an activator of protein C is added to cause the fibrin polymerization to be sensitive to the protein C pathway.

192. The method according to claim 191, wherein the protein C activator is purified human thrombomodulin, purified non-human mammalian thrombomodulin, soluble or membrane associated thrombomodulin, native thrombomodulin or thrombomodulin reconstituted with phospholipids, partially or fully glycolsylated thrombomodulin or fully deglycosylated thrombomodulin.

193. The method of claim 188, wherein the activator comprises recombinant or purified tissue factor, truncated tissue factor, or cells expressing tissue factor on their surface.

194. The method of claim 188, wherein the fibrin polymerization is monitored over time to provide a time-dependent measurement profile.

195. The method of claim 194, wherein an endpoint is extracted from the time-dependent measurement profile.

196. The method of claim 195, wherein the endpoint is normalized by using a model.

197. The method of claim 196, wherein the model is a ratio or difference of the endpoint compared to an endpoint from a time-dependent measurement profile for a known sample.

198. The method of claim 197, wherein the endpoint is initiation of clot formation, overall change in the profile, or slope of the profile after initiation of clot formation.

199. The method according to claim 194, wherein at least two time-dependent fibrin polymerization profiles are obtained, an additional profile being obtained for a known sample from computer memory or by adding said activator at at least one concentration to a known sample and monitoring the formation of fibrin polymerization over time.

200. The method according to claim 199, wherein at least one parameter from each time-dependent fibrin polymerization profile having varying activator concentrations is determined and a concentration at which the at least one parameter of said sample being tested deviates from normal is determined.

201. The method according to claim 195, wherein the endpoint is time index or value of the minimum of the first derivative, the time index or value for the minimum or maximum of the second derivative, or the overall magnitude of change.

202. The method according to claim 194, wherein the rate or acceleration of fibrin polymerization is determined from the time-dependent measurement profile, wherein said rate or acceleration is compared to rate or acceleration at the same activator concentration for a known sample and/or the rate or acceleration of the test sample at a different activator concentration.

203. The method of claim 191, wherein a fibrin polymerization profile is obtained with and without a protein C activator.

204. The method of claim 203, wherein a fibrin polymerization profile is obtained at multiple concentrations of said activator which triggers thrombin explosion.

205. The method of claim 204, wherein a fibrin polymerization profile is obtained at multiple concentrations for a known sample.

206. A method for evaluating the efficacy of an antithrombotic or procoagulant pharmaceutical, comprising:

providing a first test sample from a human or non-human mammal;

adding an activator to said first test sample in order to trigger a thrombin explosion dependent upon the propagation phase and amplification loops of the coagulation system in the test sample;

measuring fibrin polymerization in the first test sample due at least in part to said thrombin explosion;

determining whether the sample is hypocoagulable, normal or hypercoagulable, or providing a baseline;

administering one or more antithrombotic or procoagulant pharmaceuticals to the mammal;

providing at least one additional sample from the mammal at a time after administration of the pharmaceutical;

adding said activator to said at least one additional sample in order to trigger a thrombin explosion dependent upon the propagation phase and amplification loops of the coagulation system in the test sample;

measuring fibrin polymerization in said at least one additional sample due at least in part to said thrombin explosion;

determining the degree of hypocoagulability or hypercoagulability of the second mammalian sample, or a change from baseline; and

determining the efficacy of the pharmaceutical based on any changes in the hypocoagulability or hypercoagulability from the first test sample, or any changes from baseline.

207. The method of claim 206, further comprising adding vesicles to the test sample.

208. The method of claim 207, wherein the vesicles comprise platelets, cellular debris, phospholipid vesicles or platelet microparticles.

209. The method of claim 206, wherein an activator of protein C is added to cause the fibrin polymerization to be sensitive to the protein C pathway.

210. The method according to claim 209, wherein the protein C activator is purified human thrombomodulin, purified non-human mammalian thrombomodulin, soluble or membrane associated thrombomodulin, native thrombomodulin or thrombomodulin reconstituted with phospholipids, partially or fully glycolsylated thrombomodulin or fully deglycosylated thrombomodulin.

211. The method of claim 206, wherein the activator comprises recombinant or purified tissue factor, truncated tissue factor, or cells expressing tissue factor on their surface.

212. The method of claim 206, wherein the fibrin polymerization is monitored over time to provide a time-dependent measurement profile.

213. The method of claim 212, wherein an endpoint is extracted from the time-dependent measurement profile.

214. The method of claim 213, wherein the endpoint is normalized by using a model.

215. The method of claim 214, wherein the model is a ratio or difference of the endpoint compared to an endpoint from a time-dependent measurement profile for a known sample.

216. The method of claim 215, wherein the endpoint is initiation of clot formation, overall change in the profile, or slope of the profile after initiation of clot formation.

217. The method according to claim 212, wherein at least two time-dependent fibrin polymerization profiles are obtained, an additional profile being obtained for a known sample from computer memory or by adding said activator at at least one concentration to a known sample and monitoring the formation of fibrin polymerization over time.

218. The method according to claim 217, wherein at least one parameter from each time-dependent fibrin polymerization profile having varying activator concentrations is determined and a concentration at which the at least one parameter of said sample being tested deviates from normal is determined.

219. The method according to claim 213, wherein the endpoint is time index or value of the minimum of the first derivative, the time index or value for the minimum or maximum of the second derivative, or the overall magnitude of change.

220. The method according to claim 212, wherein the rate or acceleration of fibrin polymerization is determined from the time-dependent measurement profile, wherein said rate or acceleration is compared to rate or acceleration at the same activator

concentration for a known sample and/or the rate or acceleration of the test sample at a different activator concentration.

221. The method of claim 209, wherein a fibrin polymerization profile is obtained with and without a protein C activator.

222. The method of claim 221, wherein a fibrin polymerization profile is obtained at multiple concentrations of said activator which triggers thrombin explosion.

223. The method of claim 222, wherein a fibrin polymerization profile is obtained at multiple concentrations for a known sample.

224. The method of claim 207, wherein a part of the time dependent profile for each sample is compared to the same part of a time dependent measurement profile for a known sample.

225. A method comprising:
providing a plasma or whole blood sample from a first patient;
adding one or more reagents for activating coagulation, and a metal cation or metal salt which dissociates into a metal cation, and vesicles;
determining that the patient is hypercoagulable or hypocoagulable;
providing a plasma or whole blood sample from a second patient;
adding the one or more reagents comprising the same coagulation activator, metal cation or metal salt, and vesicles as in step (b) to the second patient sample;
determining that the second patient is the other of hypocoagulable or hypercoagulable opposite to the first patient.

226. A method for assessing the hemostatic potential of a sample comprising:

a. providing a sample to be tested;
b. adding a coagulation activator to the sample;

c. generating a time dependent measurment profile; and
d. assessing the hemostatic potential of the sample from the time dependent measurement profile.

227. The method of claim 226, further comprising determining whether the sample is hypocoagulable, normal or hypercoagulable based on the assessed hemostatic potential.

228. The method of claim 226, further comprising determining whether a patient from whom the sample was taken has a thrombotic or hemorhagic tendency.

229. The method according to claim 226, wherein all or part of said time-dependent profile is compared to all or part of a time-dependent profile for a known sample.

230. The method according to claim 229, wherein part of said profile is compared, said part of said profile including one or more of initiation of clot formation, overall change in profile, slope of profile after initiation of clot formation, and acceleration at the time of clot initiation.

231. The method according to claim 229, wherein at least two time-dependent fibrin polymerization profiles are obtained, an additional profile being obtained for a known sample from computer memory or by adding said activator at at least one concentration to a known sample and monitoring the formation of fibrin polymerization over time.

232. The method according to claim 231, wherein at least two time-dependent fibrin polymerization profiles are obtained, one profile for said test sample at a first activator concentration, and at least one additional profile for said test sample at a second

activator concentration and/or one or more profiles for a known sample at one or more activator concentrations.

233. The method according to claim 226, wherein the activator comprises tissue factor.

234. The method according to claim 231, wherein at least one parameter from each time-dependent fibrin polymerization profile having varying activator concentrations is determined and a concentration at which the at least one parameter of said sample being tested deviates from normal is determined.

235. The method according to claim 234, wherein said at least one parameter is selected from time index and value of the minimum of the first derivative, the time index and value for the minimum and maximum of the second derivative and the overall magnitude of change.

236. The method according to claim 232, wherein part of each fibrin polymerization profile is compared to a same part of a profile for a known sample.

237. The method according to claim 236, wherein said part is one or more of a time index of the minimum of the first derivative, the value of the minimum of the first derivative, the time index for the minimum of the second derivative, the value for the minimum of the second derivative, the time index of the maximum of the second derivative, the value of the maximum of the second derivative, and the overall magnitude of change.

238. The method according to claim 236, wherein said part is rate or acceleration of fibrin polymerization, wherein said rate or

acceleration is compared to rate or acceleration at the same activator concentration for said known sample.

239. The method according to claim 236, wherein a difference or ratio of said parameters for said test sample and said normal sample are determined.

240. A method comprising:
providing a test sample from the patient;
initiating coagulation in the sample in the presence of a coagulation activator and optionally an activator of an anticoagulant pathway, the coagulation activator added to the sample in an amount which will result in intrinsic tenase-dependent fibrin polymerization;
monitoring formation of said intrinsic tenase-dependent fibrin polymerization over time so as to derive a time-dependent profile;
looking at an endpoint from the time-dependent profile to assess the hemostatic potential of the test sample.

241. The method of claim 240, further comprising:
repeating steps a) to d) but changing the concentration of the coagulation activator, changing the concentration of the activator of an anticoagulant pathway, and/or changing the endpoint.

242. The method of claim 241, wherein step e) is performed when the first patient sample is hypercoagulable or hypocogulable.

243. The method of claim 242, wherein step e) is performed when the first patient sample is mildly hypercoagulable or hypocoagulable.

244. The method of claim 240 performed on an automated coagulation analyzer.

245. The method of claim 244, wherein the time dependent profile is provided by monitoring light absorbance or transmittance through a cuvette.

246. The method of claim 241, wherein the coagulation activator is tissue factor, the anticoagulant pathway activator is thrombomodulin, and the endpoint is selected from a time index of the minimum of the first derivative, the value of the minimum of the first derivative, the time index for the minimum of the second derivative, the value for the minimum of the second derivative, the time index of the maximum of the second derivative, the value of the maximum of the second derivative, and the overall magnitude of change.

247. The method of claim 241, wherein the endpoint is other than clot time.

248. The method of claim 241, wherein more than one of the concentration of the coagulation activator, the concentration of the activator of an anticoagulant pathway, and the endpoint are altered in step e).

249. The method of claim 241, wherein the endpoint is initiation of clot formation, overall change in the time dependent profile, slope of the profile after initiation of clot formation, and/or acceleration at the time of clot initiation.

250. The method of claim 240, wherein the endpoint is a variable within a curve fit function.

251. The method of claim 188, wherein the fibrin polymerization measurement is used to adjust the patient's therapy to result in a fibrin polymerization profile approximating normal.

252. A method for assessing the hemostatic potential of a sample, comprising:

adding to a sample a coagulation activator, phospholipid vesicles, metal ions or metal salt if the sample is citrated, and optionally an activator of an anticoagulant pathway;

monitoring the polymerization of fibrin in the sample; and

assessing the hemostatic potential of the sample based on the kinetics of the fibrin polymerization;

wherein the coagulation activator is tissue factor sufficiently diluted so as to result in an approximately 0.75 to 3.0 pico molar concentration range when the reagent is mixed with the sample.

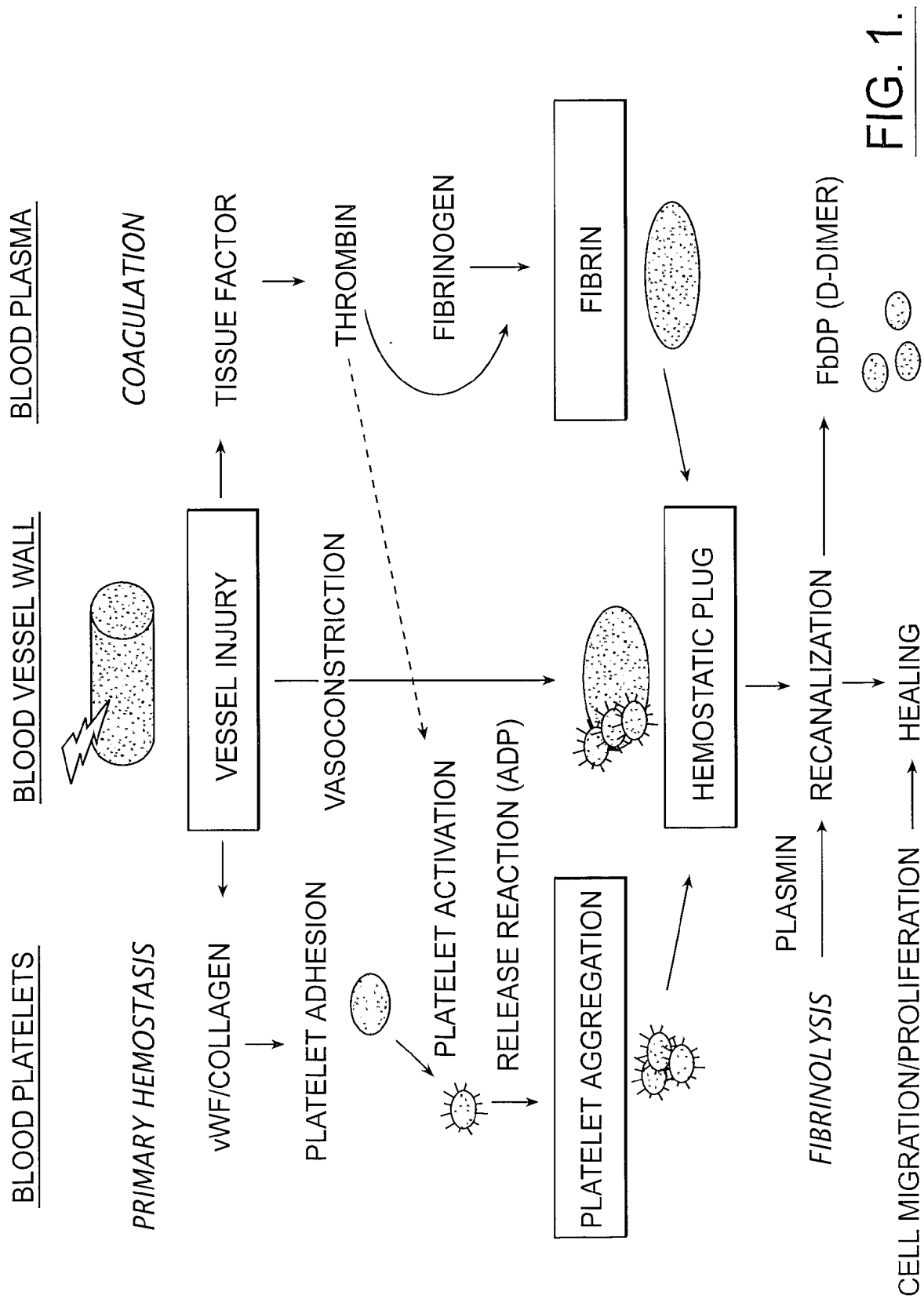
BLOOD PLATELETS
BLOOD VESSEL WALL
BLOOD PLASMA
PRIMARY HEMOSTASIS
COAGULATION
VWF/COLLAGEN
VESSEL INJURY
TISSUE FACTOR
THROMBIN
FIBRINOGEN
FIBRIN
PLATELET ADHESION
VASOCONSTRICTION
PLATELET ACTIVATION
RELEASE REACTION (ADP)
PLATELET AGGREGATION
HEMOSTATIC PLUG
PLASMIN
RECANALIZATION
FbDP (D-DIMER)
FIBRINOLYSIS
HEALING
CELL MIGRATION/PROLIFERATION

FIG. 1.

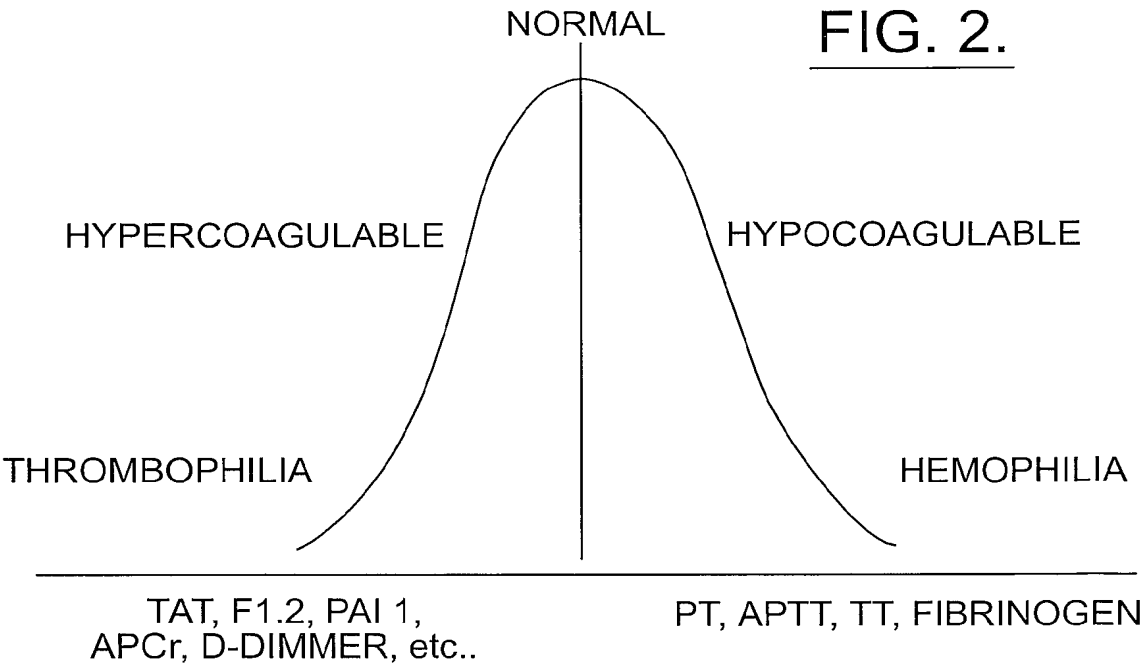
NORMAL
HYPERCOAGULABLE
HYPOCOAGULABLE
THROMBOPHILIA
HEMOPHILIA
TAT, F1.2, PAI 1,
APCr, D-DIMMER, etc..
PT, APTT, TT, FIBRINOGEN

FIG. 2.

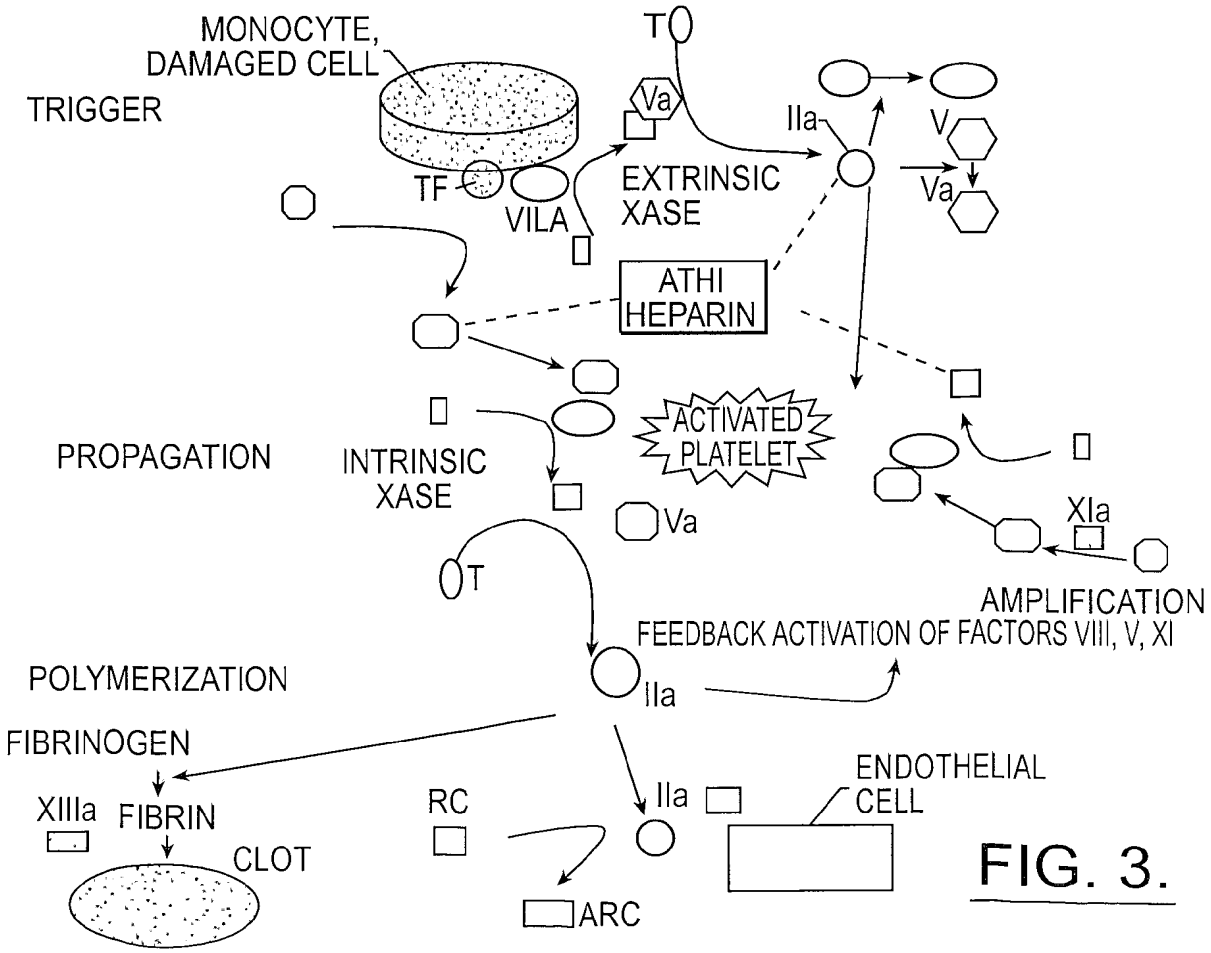
MONOCYTE,
DAMAGED CELL
TRIGGER
TF
VILA
T
Va
EXTRINSIC
XASE
IIa
V
Va
ATHI
HEPARIN
PROPAGATION
INTRINSIC
XASE
ACTIVATED
PLATELET
Va
XIa
T
AMPLIFICATION
FEEDBACK ACTIVATION OF FACTORS VIII, V, XI
POLYMERIZATION
IIa
FIBRINOGEN
XIIIa
FIBRIN
CLOT
RC
IIa
ENDOTHELIAL
CELL
ARC

FIG. 3.

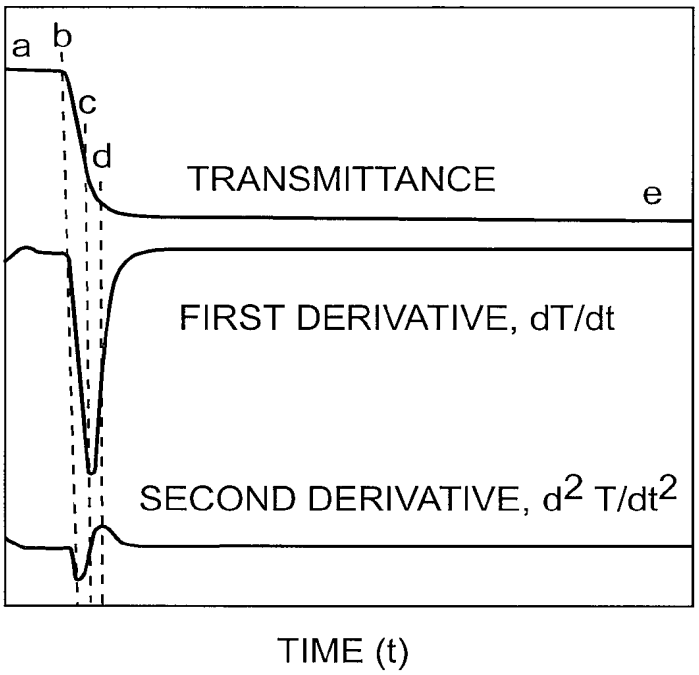
a
b
c
d
TRANSMITTANCE
e
FIRST DERIVATIVE, dT/dt
SECOND DERIVATIVE, d2 T/dt2
TIME (t)

FIG. 4.

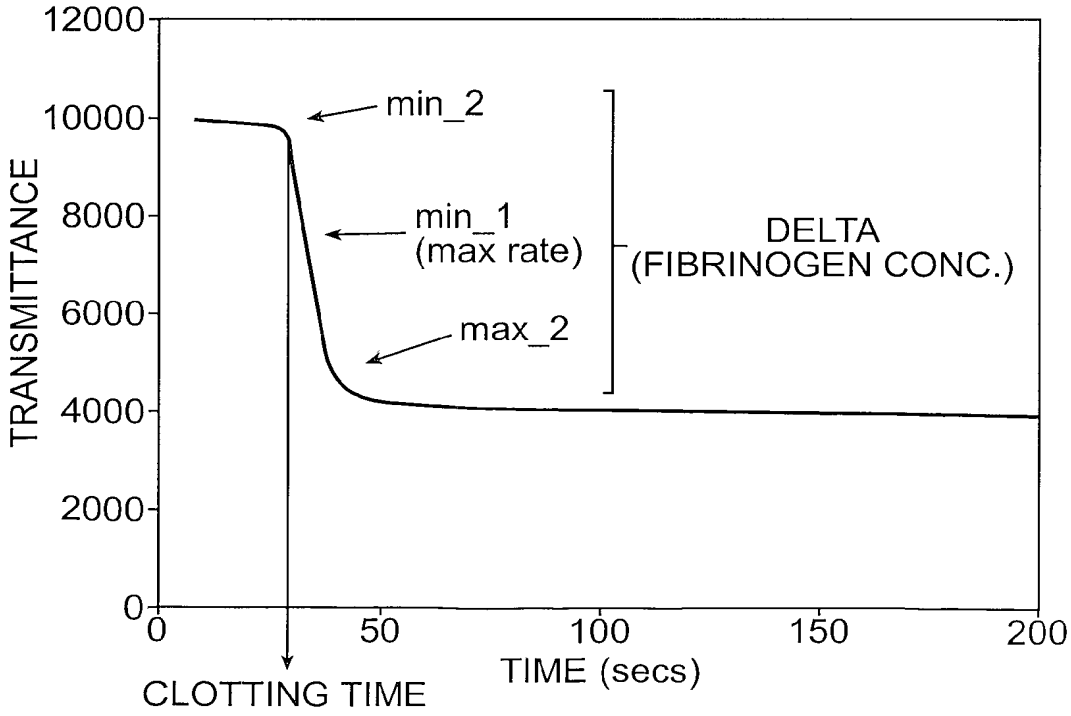
12000
10000
8000
6000
4000
2000
0
TRANSMITTANCE
min_2
min_1
(max rate)
max_2
DELTA
(FIBRINOGEN CONC.)
0
50
100
150
200
TIME (secs)
CLOTTING TIME

FIG. 5.

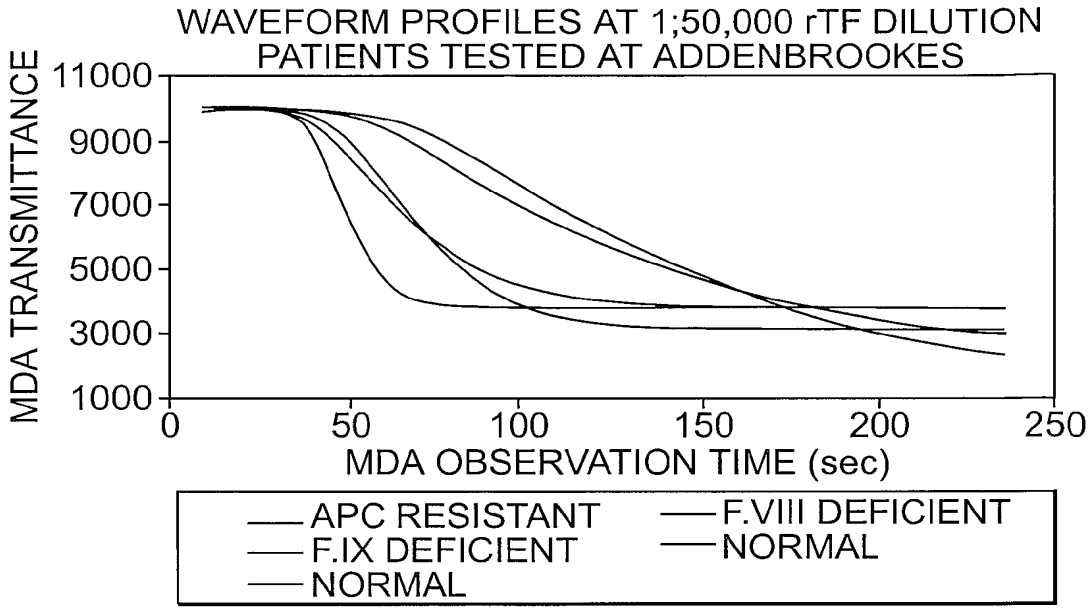
WAVEFORM PROFILES AT 1;50,000 rTF DILUTION
PATIENTS TESTED AT ADDENBROOKES
MDA TRANSMITTANCE
11000
9000
7000
5000
3000
1000
0
50
100
150
200
250
MDA OBSERVATION TIME (sec)
APC RESISTANT
F.IX DEFICIENT
NORMAL
F.VIII DEFICIENT
NORMAL

FIG. 6.

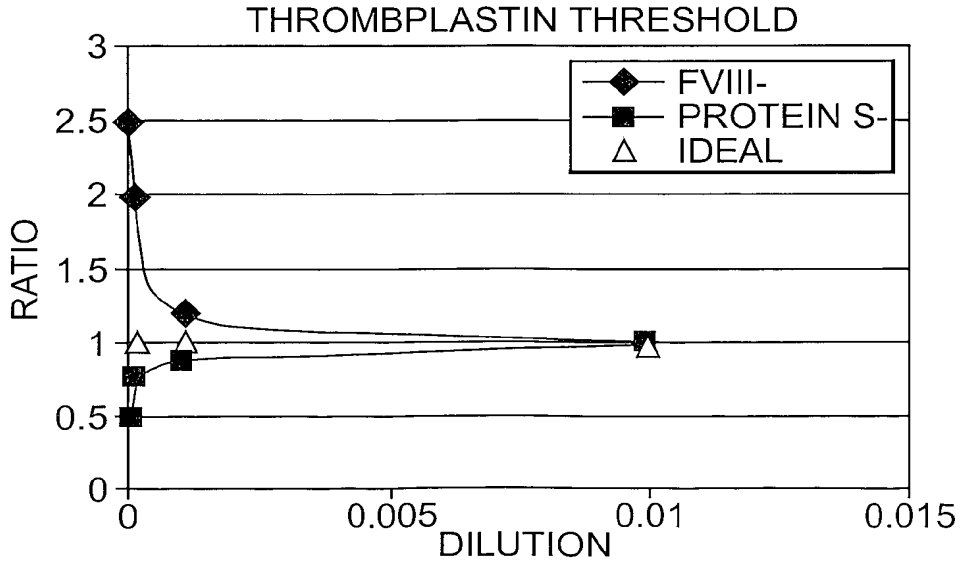
THROMBPLASTIN THRESHOLD
RATIO
3
2.5
2
1.5
1
0.5
0
0
0.005
0.01
0.015
DILUTION
FVIII-
PROTEIN S-
IDEAL

FIG. 7.

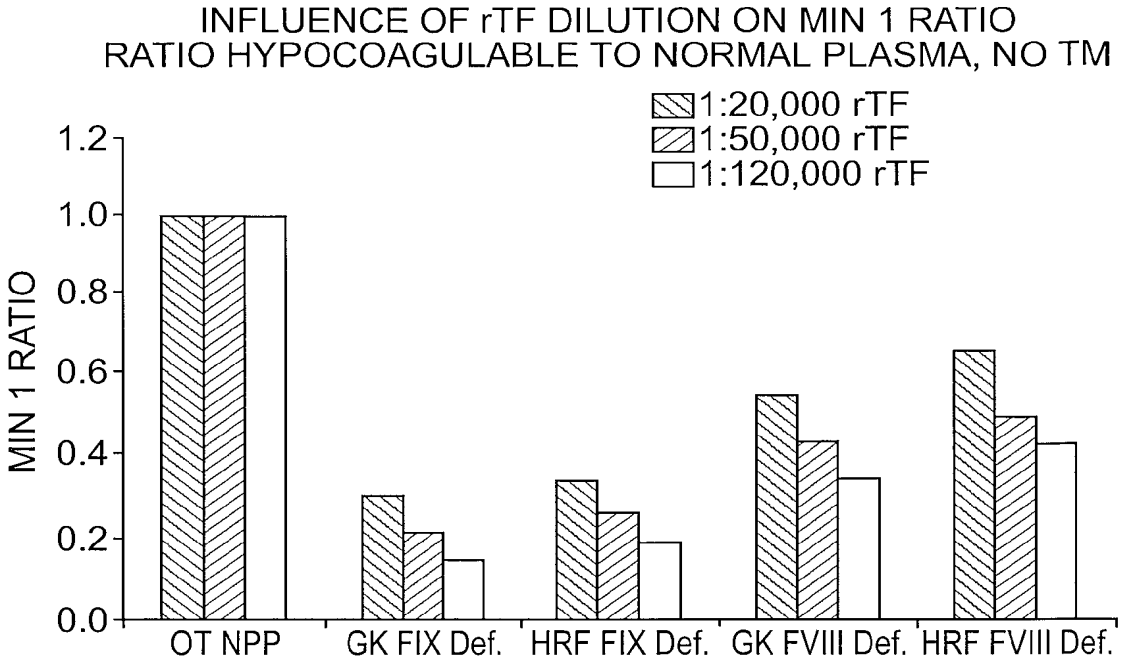
INFLUENCE OF rTF DILUTION ON MIN 1 RATIO
RATIO HYPOCOAGULABLE TO NORMAL PLASMA, NO TM
1:20,000 rTF
1:50,000 rTF
1:120,000 rTF
MIN 1 RATIO
1.2
1.0
0.8
0.6
0.4
0.2
0.0
OT NPP
GK FIX Def.
HRF FIX Def.
GK FVIII Def.
HRF FVIII Def.

FIG. 8.

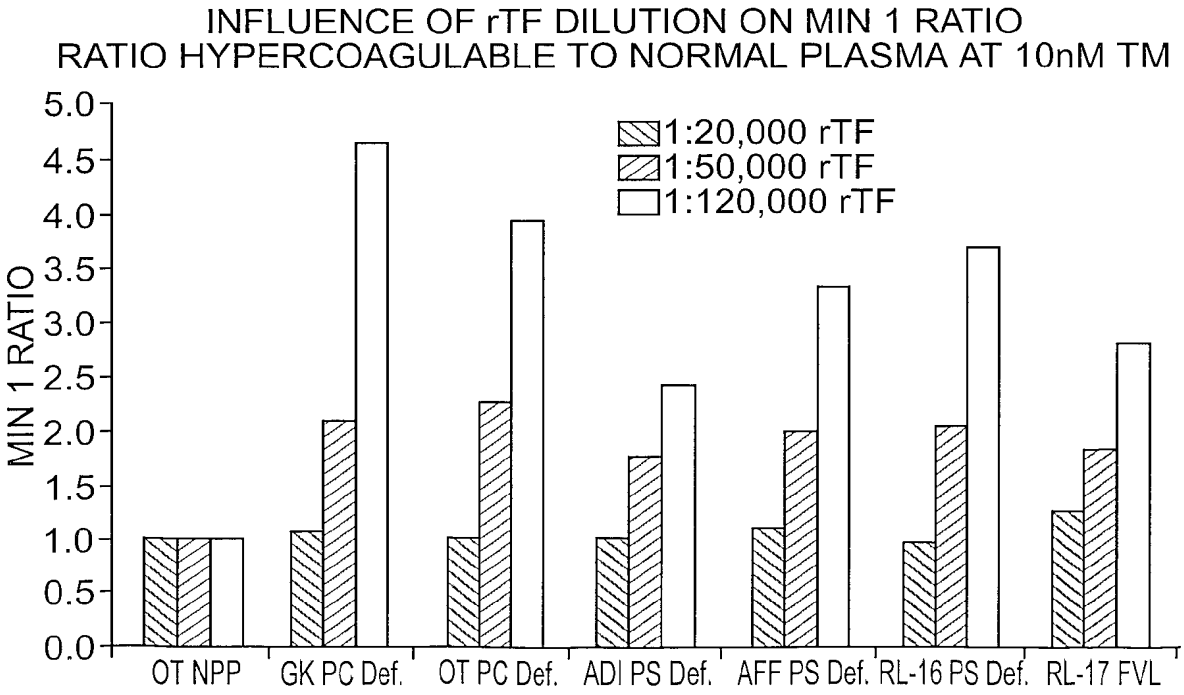
INFLUENCE OF rTF DILUTION ON MIN 1 RATIO
RATIO HYPERCOAGULABLE TO NORMAL PLASMA AT 10nM TM
1:20,000 rTF
1:50,000 rTF
1:120,000 rTF
MIN 1 RATIO
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
OT NPP
GK PC Def.
OT PC Def.
ADI PS Def.
AFF PS Def.
RL-16 PS Def.
RL-17 FVL

FIG. 9.

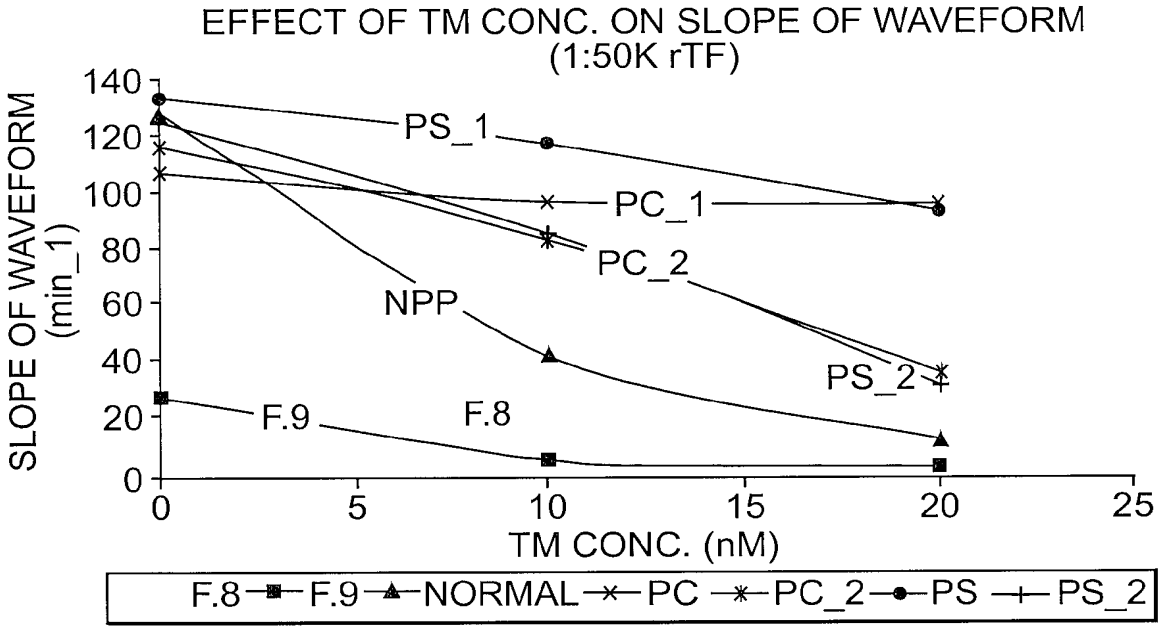
EFFECT OF TM CONC. ON SLOPE OF WAVEFORM
(1:50K rTF)
SLOPE OF WAVEFORM (min_1)
140
120
100
80
60
40
20
0
0
5
10
15
20
25
TM CONC. (nM)
PS_1
PC_1
PC_2
NPP
PS_2
F.9
F.8
F.8 F.9 NORMAL PC PC_2 PS PS_2

FIG. 10.